JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, January 2011, Vol. 56, No. 1
doi: 10.1111/j.1556-4029.2010.01545.x
Available online at: interscience.wiley.com

CASE REPORT

ODONTOLOGY

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Comparison of Age Estimation in Japanese and Scandinavian Teeth Using Amino Acid Racemization*

ABSTRACT: Racemization method is currently considered to be one of the most accurate methods for age estimation. There are many factors that affect racemization reaction velocities. We investigated ethnic differences in the racemization reaction velocities between Japanese and Scandinavian teeth. After a heating experiment, the Arrhenius equation was used to obtain a rate constant (k). Both groups presented almost identical velocities, indicating that there was no difference in the tooth racemization reaction between the two groups. However, because 14 of the 18 Scandinavian teeth had multiple roots, it is possible that accurate racemization rates of the teeth themselves were not reflected in the results. These findings reconfirm that the type of tooth selected is extremely important when evaluating age by racemization.

KEYWORDS: forensic science, age estimation, teeth, racemization, D-aspartic acid, dentin

D-Amino acids are produced during the course of aging by racemization of L-amino acids, and of all the amino acids, aspartic acid has the highest racemization reaction rate (1). D-Aspartic acid is accumulated in slowly metabolizing tissues, for example, teeth. Racemization is a first-order chemical reaction. Therefore, the velocity of the racemization reaction is enormously influenced by temperatures.

Measuring the racemization of aspartic acid in teeth is currently considered to be the most accurate method of estimating age and widely used (2–6). Dentin samples are used in this technique because of dentin's stable environment (owing to being surrounded by enamel at the crown and by cementum at the root) and its constant water content maintained by the dentin tubules. In addition, the process of collecting 5–10 mg of dentin from the entire dentin of 1-mm-thick longitudinal sections has been shown to yield reliable results (7). We advocate the use of single-rooted teeth, such as mandibular incisors or mandibular premolars (8) because all the dentin can be easily collected, and the test results obtained from single-rooted teeth are considered to reflect racemization rates of the dentin itself.

As a control, several matching teeth from individuals of known age are required to obtain an accurate age estimation (6). The control teeth are used to obtain an equation deduced from the least square method using racemization rates from the control teeth and the actual ages.

Although this study examined teeth from Scandinavian people, we used single-rooted teeth from Japanese people as a control without matching tooth types, because of the relatively large number of samples and the lack of availability of teeth from the Swedish population. Therefore, the results were not as accurate as in previous Japanese studies. We speculated that the velocity of the racemization reaction of aspartic acid might vary depending upon ethnic group. The reasons for the inaccuracy were examined by determining the velocity of the racemization reaction in Japanese and Scandinavian teeth by conducting a heating experiment to calculate the Arrhenius equation (9).

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*Supported by the National Institute on Drug Abuse, Contract No. N01DA-5-7746. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Received 5 Aug. 2009; and in revised form 1 Oct. 2009; accepted 25 Oct. 2009.

Materials and Methods

Eleven single-rooted teeth from Japanese people were used as a control in the age estimation of 18 teeth from Scandinavian people (four single-rooted teeth and 14 multiple-rooted teeth). We prepared longitudinal sections (about 1-mm thickness), carefully dissected out dentin from the sections, and measured the racemization rates of aspartic acid using gas chromatography as in our previous study (10). These findings were used to calculate the Arrhenius equation. Uniformly powdered dentin (74–297 μm) was then used in a heating experiment using Japanese and Swedish teeth. Each 5 mg of sample was hermetically sealed in a screw-top test tube and heated with an aluminum block heater. The samples were heated at 120°C, 130°C, or 150°C for 1, 3, 5, or 7 h to obtain racemization velocity constants (k).

Results and Discussion

To determine the age of the Scandinavian teeth, the following equation for age estimation was derived from 11 single-rooted Japanese teeth (Table 1, Fig. 1):

$$Y = 783.81X - 28.18$$

where Y is age and X is $\ln[(1+D/L)/(1-D/L)]$.

TABLE 1—*Age estimation in teeth by racemization of aspartic acid.*

						An Equation for Age Estimation in Japanese Teeth		An Equation for Age Estimation in Scandinavian Teeth	
						$Y = 783.81X - 28.18$, $n = 11$		$Y = 778.80X - 27.15$, $n = 18$	
	No.	Teeth	Root	Actual Age	D/L	Age Estimation	Error	Age Estimation	Error
S	1	Mandibular central incisor	S	59.4	0.1074	56.0	3.4	56.5	2.9
	2	Maxillary first premolar	M	60.7	0.1022	51.9	8.8	52.4	8.3
	3	Maxillary first premolar	M	60.7	0.1048	54.0	6.7	54.5	6.2
	4	Mandibular second molar	M	44.1	0.0984	48.9	−4.8	49.5	−5.4
	5	Mandibular second molar	M	44.1	0.0908	43.0	1.1	43.6	0.5
	6	Maxillary first premolar	M	16.3	0.0534	13.7	2.6	14.4	1.9
	7	Maxillary first premolar	M	18.7	0.0610	19.6	−0.9	20.4	−1.7
	8	Maxillary canine	S	70.6	0.1228	68.1	2.5	68.5	2.1
	9	Maxillary first premolar	M	15.5	0.0552	15.1	0.4	15.8	−0.3
	10	Mandibular second molar	M	18.1	0.0614	19.9	−1.8	20.7	−2.6
	11	Mandibular second molar	M	13.4	0.0496	10.7	2.7	11.5	1.9
	12	Mandibular second molar	M	17.8	0.0566	16.2	1.6	16.9	0.9
	13	Mandibular second molar	M	64.6	0.1196	65.6	−1.0	66.0	−1.4
	14	Mandibular central incisor	S	68.2	0.1238	68.9	−0.7	69.3	−1.1
	15	Maxillary lateral incisor	S	51.0	0.1044	53.6	−2.6	54.2	−3.2
	16	Mandibular second molar	M	46.6	0.1046	53.8	−7.2	54.3	−7.7
	17	Maxillary first premolar	M	13.8	0.0490	10.2	3.6	11.0	2.8
	18	Maxillary first premolar	M	16.1	0.0610	19.6	−3.5	20.4	−4.3
				$r = 0.984$		SE = ±0.93		SE = ±0.93	
						SD = ±3.94		SD = ±3.96	
J	1	Mandibular first premolar	S	21	0.0636	21.6	−0.6	22.4	−1.4
	2	Mandibular second premolar	S	39	0.0892	41.7	−2.7	42.3	−3.3
	3	Mandibular first premolar	S	46	0.0914	43.5	2.5	44.0	2.0
	4	Mandibular central incisor	S	54	0.1066	55.4	−1.4	55.9	−1.9
	5	Mandibular first premolar	S	54	0.1034	52.9	1.1	53.4	0.6
	6	Maxillary first premolar	S	56	0.1074	56.0	0	56.5	−0.5
	7	Mandibular first premolar	S	62	0.1128	60.2	1.8	60.7	1.6
	8	Maxillary first premolar	S	62	0.1144	61.5	0.5	61.9	0.1
	9	Mandibular central incisor	S	62	0.1124	59.9	2.1	60.4	1.6
	10	Mandibular central incisor	S	66	0.1204	66.2	−0.2	66.6	−0.6
	11	Mandibular central incisor	S	67	0.1254	70.1	−3.1	70.5	−3.5
				$r = 0.991$		SE = ±0.56		SE = ±0.60	
						SD = ±1.87		SD = ±1.97	

S, Scandinavian teeth; J, Japanese teeth; *Y*, age; *X*, ln[(1+D/L)/(1-D/L)]; SE, standard error; SD, standard deviation; D/L, ln[(1+D/L)/(1-D/L)]; *r*, coefficient of correlation; S, single-rooted teeth; M, multiple-rooted teeth.

The results obtained were not accurate: seven of 18 cases exceeded the limit of errors by more than ±3 years (SD = ±3.94). Six cases exhibited the same trend (SD = ±3.96) when an equation derived from Scandinavian teeth ($Y = 778.80X - 27.15$) was used (Table 1, Fig. 1).

Of the 11 Japanese teeth used as a control (Table 1), the limit of errors was exceeded in one case where the equation derived from the Japanese teeth was applied (SD = ±1.87) and in two cases where the equation derived from the Scandinavian teeth was applied (SD = ±1.97). These findings suggest that the inaccuracy in age estimation we observed may be the result of variations in racemization velocities according to ethnic group.

To test this hypothesis, racemization reaction velocities of Japanese and Swedish teeth were compared by conducting a heating experiment.

The Arrhenius equation has been widely used because Arrhenius (9) confirmed quantitative relations existing between reaction temperature and velocity. The Arrhenius equation is expressed as a straight line obtained by plotting logarithms (link) of reaction velocity constants (k) against reciprocal numbers of absolute temperature (l/T) (9).

The racemization rate of aspartic acid increased linearly at different velocities depending on the temperature and time after heating the dentin powder (Fig. 2). Arrhenius equations derived from the velocity constants (k) of the equations for the racemization reaction at each temperature for Japanese teeth (lnk(h) = −7520.01(1/T) + 12.5622, $r = 0.999$) and Scandinavian teeth (lnk(h) = −7535.90 (1/T) + 12.5561, $r = 0.999$) are shown in Fig. 3. Subsequently, k (*y*) values of each group were obtained at environmental temperature (16°C). The results reveal that the racemization reaction rate velocities were almost the same (Japanese: k (*y*) = 0.0127, Scandinavian: k (*y*) = 0.0119), indicating that there is almost no difference in the tooth substance of the two groups as evidenced by racemization reaction. In accordance with these results, Rits-Timme et al. (11) also reported that there was no difference between Turkish and German races in view of aspartic acid racemization in dentin.

Dentin formation commences at the junction between the enamel and dentin and progresses in the direction of the pulp and the apex. The completion of dentin formation takes 8–10 years or longer depending on the tooth type and the individual (12). Thus, variations in the racemization rate exist depending on the location of the dentin, possibly resulting in a higher racemization rate in the crown portion and a lower rate in the root. However, in older age groups, these results are reversed because of the long time period after completion of dentin formation, and the racemization rates of the

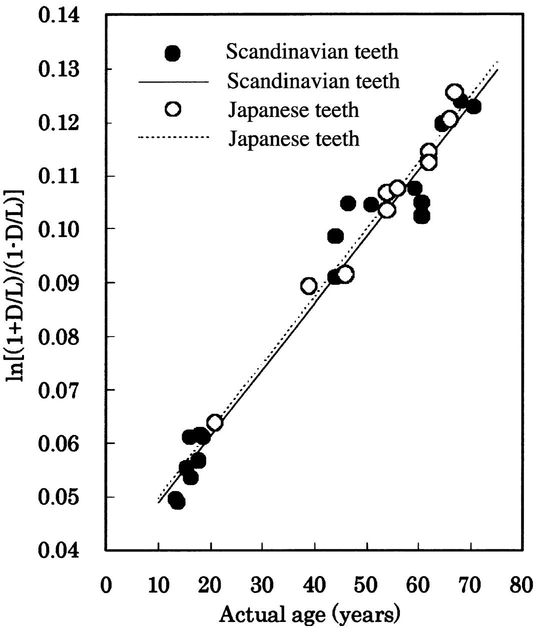

FIG. 1—*Relationships between racemization rates of Japanese teeth and actual age, and between racemization rates of Scandinavian teeth and actual age.*

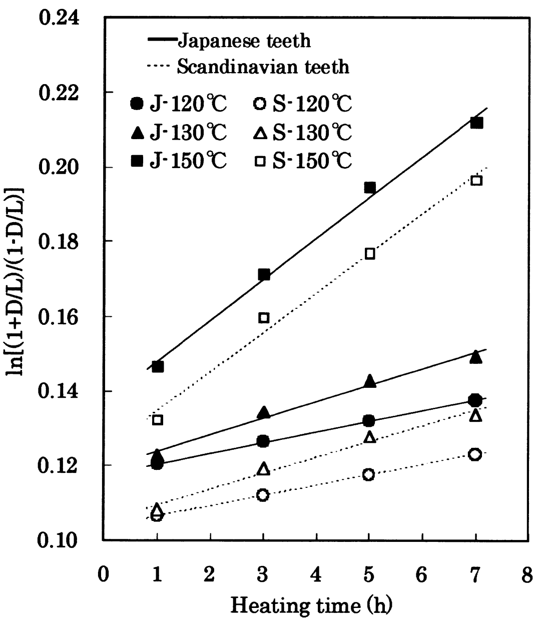

FIG. 2—*Changes in racemization rate of aspartic acid on heating. S, Scandinavian teeth; J, Japanese teeth.*

roots tend to be affected by environmental temperature (13). Therefore, we collected 5–10 mg of powdered dentin mixed from the crown and root dentin sample after fabrication of longitudinal tooth sections (7). Racemization rates of the mixed dentin of crown and root were obtained through this procedure. Single-rooted teeth, such as mandibular incisors or mandibular premolars, are the most desirable tooth types to obtain the racemization rate of the dentin. However, in our study, the samples included seven maxillary first premolars and seven mandibular second molars.

These results demonstrate that accurate age estimation was not achieved in this study because the racemization rates of the dentin

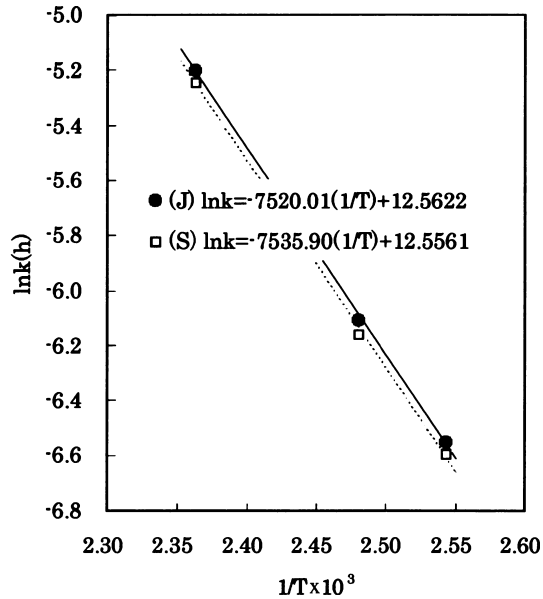

FIG. 3—*Arrhenius plot. S, Scandinavian teeth; J, Japanese teeth.*

TABLE 2—*Racemization rates in the first molar and estimated ages.*

Teeth	Type of Root	Actual Age	D/L	Age Estimation	Error
Maxillary first molar (right)	Multiple	32	0.0832	32	0
Maxillary first molar (right)	Multiple	38	0.0956	42	4
Maxillary first molar (left)	Multiple	42	0.0894	37	−5
Maxillary first molar (left)	Multiple	44	0.0950	42	−2
Maxillary first molar (right)	Multiple	45	0.0994	45	0
Maxillary first molar (left)	Multiple	45	0.0982	44	−1
Maxillary first molar (left)	Multiple	53	0.1056	50	−3
Maxillary first molar (right)	Multiple	53	0.1108	55	−3
Maxillary first molar (right)	Multiple	59	0.1164	59	0
Maxillary first molar (left)	Multiple	62	0.1194	61	−1
Maxillary first molar (left)	Multiple	66	0.1228	64	−2

$Y = 800.51X - 33.66$; $r = 0.975$; $n = 11$ {D/L, ln[(1+D/L)/(1−D/L)]; Y, age; X, ln[(1+D/L)/(1−D/L)]}.

itself were not accurate because of the inclusion of a number of multiple-rooted maxillary and mandibular molars, rather than ethnic differences. To confirm this speculation, we further measured racemization rates of aspartic acid using Japanese multiple-rooted teeth (maxillary first molars) and estimated ages from the deduced regression line (Table 2). As we expected, the accuracy of estimated ages was lower ($r = 0.975$) than using single-rooted teeth (6). These conclusions reconfirm that the type of teeth selected is extremely important for age evaluation by racemization.

Acknowledgments

We thank Dr. Kirsty L. Spalding of the Karolinska Institute (CMB, Department of Cell and Molecular Biology, Medical Nobel Institute) for providing Scandinavian teeth. The authors appreciate the cooperation of the DEA regional laboratories in submitting all the nondomestic samples and the states' eradication programs for submitting the domestic samples.

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