Calibration of Fractionated Metanephrines in Urine: Still an Issue?

To the Editor:

Measurement of fractionated total metanephrines in urine provides important information for the diagnosis of pheochromocytoma (1). Reference intervals that distinguish patients who harbor a pheochromocytoma from those with similar symptoms not actually caused by this disease are highly variable (2–4). Such differences may be caused by variations in analytical methods, the selection of negative controls for establishing reference values, age, and sex (4). A group of 3 laboratories have reported in this journal significantly higher results than expected for urinary fractionated total metanephrines during 3 successive proficiency surveys in 2003 and 2004. These differences were due to assigned values for the Bio-Rad Laboratories calibrator that were 24%–33% lower than the actual concentration (5). To determine whether the concentration of the Bio-Rad calibrator that has been corrected, we examined proficiency-test data obtained from ProBioQual, a French quality-assurance center, to assess the accuracy of urine metanephrine results collected from 48 laboratories during the 2004–2006 period. We included only 8 surveys with target metanephrine concentrations between 1000 and 1800 nmol/L in this retrospective analysis to exclude the uncertainty produced by measurements of very low or very high concentrations. Forty-eight laboratories were requested to specify the calibrator used, and 34 laboratories actually returned this information. Ten laboratories used an in-house calibrator, and calibrators manufactured by Chromsystems Instruments & Chemicals (Munich, Germany), Bio-Rad, and Recipe Chemicals + Instruments (Munich, Germany) were used by 15, 7, and 2 laboratories, respectively. An ANOVA of the results for the 8 surveys showed no statistical differences, with the exceptions of survey no. 6 in 2005, which revealed higher normetanephrine concentrations in laboratories that used Chromsystems reagents than in those that used an in-house calibrator (difference, 15.6%; \( P = 0.048 \)), and survey no. 3 in 2006, which found higher metanephrine concentrations in laboratories that used Chromsystems and Bio-Rad calibrators than in those that used an in-house calibrator (differences of 9% and 15.4%, respectively; \( P = 0.005 \)). The number of users of Recipe calibrators was too low (0–2) to evaluate. Despite the differences in these 2 surveys, we concluded that the metanephrine concentrations obtained by the laboratories were roughly similar. A runs test on variation coefficients revealed an improvement in precision for metanephrine (\( P = 0.02 \)) beginning in June 2005, but not for normetanephrine (\( P = 0.45 \)). Because of the limited number of runs, these results must certainly be interpreted with caution, but they are indicative of decreasing dispersion for metanephrine among the participating laboratories (Fig. 1).

We then determined whether the concentrations of fractionated total metanephrines measured by HPLC with amperometric detection were similar for the urine calibrator from Bio-Rad, 2 commercially available sources (Recipe and Chromsystems), and our in-house calibrator, which is prepared by weighing normetanephrine and metanephrine obtained from Sigma-Aldrich. In addition, we used the Bio-Rad calibrator to measure metanephrine concentration across all the samples to determine whether 2 concentrations of the internal quality controls (typical and abnormal) from Chromsystems and Recipe were in agreement with their nominal values.

We repeated the experiments 10 times within 1 month with freshly prepared calibrators and controls. Our results showed mean systematic departures between found and expected concentrations ranging from 91% to 100% among calibrators and internal quality controls for both metanephrines and normetanephrines, whereas the CVs observed within the 10 determinations for metanephrines ranged between 2% and 9%. An ANOVA revealed significant differences between the groups for both normetanephrine (\( P = 0.0026 \)) and metanephrine (\( P < 0.0001 \)). Post hoc Scheffé tests indicated that our in-house calibrator exhibits a 9% higher normetanephrine concentration than the Chromsystems calibrator (\( P = 0.008 \)).

The Chromsystems metanephrine calibrator exhibited 5% higher values than the Recipe calibrator (\( P < 0.0001 \)). The metanephrine concentrations observed for the Chromsystems abnormal-concentration internal quality control were also 10% higher than expected compared with those from Recipe (\( P = 0.008 \)). Despite the statistical significance of this difference (\( P = 0.0026 \)), it is not expected to affect the medical decision to a clinically relevant degree.

In conclusion, we did not notice any crucial differences between...
the Bio-Rad, Recipe, and Chromsystems calibrators for metanephrines. The increase in sources of calibrators did not cause a decrease in the accuracy of the results during external quality control program in the 2004–2006 period. The observed differences in published reference values for urinary metanephrines are not mainly because of analytical reasons but most probably are due to differences in the characteristics of reference groups. The present availability of consistent calibrators should prompt efforts to define and validate reference values based on a large pool of observations, which then could be used in multiple laboratories.

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References


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Maple Syrup Urine Disease: Newborn Screening Fails to Discriminate between Classic and Variant Forms

To the Editor:

Maple syrup urine disease (MSUD)5 (OMIM 248600) is an inborn error of metabolism detectable by newborn screening (NBS). Deficiency of the branched-chain 2-keto acid dehydrogenase leads to the accumulation of branched-chain amino acids (BCAA) leucine, valine, isoleucine, and alloisoleucine. About 75% of MSUD patients have the severe classic form (<2% enzyme activity). They develop a severe encephalopathic crisis with deep coma, mostly during the second week of life, owing to very high concentrations of BCAAs (≥1000 μmol/L leucine). The remainder have milder variant forms (2%–40% enzyme activity) with lower concentrations of BCAAs (<1500 μmol/L leucine) and later onset or absence of cerebral symptoms (1).

Severely ill newborns require urgent lowering of branched-chain compounds followed by lifelong semisynthetic diet with reduced intake of BCAAs. Because even subjects with a mild form of MSUD are at risk of acute metabolic decompensation during stressful situations, they also may benefit from early presymptomatic diagnosis by NBS. Whereas newborns with classic MSUD need emergency management including intensive care and occasionally extracorporeal detoxification, this expensive treatment is not necessary in newborns with variant MSUD. We performed the present study to find out if classic and variant MSUD can be discriminated by NBS so that adequate treatment may be initiated when receiving the result of a tentative diagnosis of MSUD in NBS.

Since 2002, electrospray ionization–tandem mass spectrometry (ESI-MS/MS)–based NBS has been available in Germany for every newborn. NBS for MSUD is performed by measuring the concentration of “total leucine” (leucine + isoleucine + alloisoleucine + hydroxyproline) in dried blood spots. These 4 isobaric amino acids cannot be separated by routine screening methods. The
tentative diagnosis has to be confirmed by measuring BCAA concentrations in plasma. The recall rate for MSUD in Germany is ~0.01% (calculated from >1.5 million newborns in the Bavarian and Berlin screening laboratory).

We compared total leucine concentrations in dried blood and leucine concentrations in plasma during confirmation diagnosis between subjects attributed retrospectively to classic or variant MSUD. Data on the patients with classic MSUD have been published recently (2). Data are presented as ranges and/or mean (SD). Data analysis was performed by SPSS-12 (SPSS Inc). The Wilcoxon rank sum test (Mann–Whitney U-test) was used to compare differences between the 2 groups of patients. \[ P < 0.05 \] was considered statistically significant.

In Table 1, we present laboratory data of 19 newborns who screened positive for MSUD in Germany and Austria from 1999 to 2005. MSUD was suspected by detecting increased total leucine concentrations in dried blood spots collected between the second and eighth day of life. Total leucine concentrations in newborns suffering from classic MSUD (subjects C1–C10) were significantly higher than concentrations in newborns who were later attributed to a variant form (subjects V1–V9) [1240 (557) \( \mu \text{mol/L} \) vs 549 (267) \( \mu \text{mol/L} \), \( P = 0.003 \)]. Because of high variance, however, it was impossible to reli-

### Table 1. Laboratory data for NBS and confirmation diagnosis in newborns who screened positive for MSUD

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blood sampling, day of life</th>
<th>Total leucine or Xle, ( \mu \text{mol/L} )</th>
<th>Val, ( \mu \text{mol/L} )</th>
<th>Xle/Phe, molar ratio</th>
<th>Xle/Ala, molar ratio</th>
<th>Confirmation of diagnosis, day of life</th>
<th>BCAA concentrations at start of treatment/confirmation of diagnosis, ( \mu \text{mol/L} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>3</td>
<td>835</td>
<td>830</td>
<td>12.3</td>
<td>5.4</td>
<td>6</td>
<td>339 (301)</td>
</tr>
<tr>
<td>V2</td>
<td>8</td>
<td>1030</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>497 (466)</td>
</tr>
<tr>
<td>V3</td>
<td>3</td>
<td>485</td>
<td></td>
<td></td>
<td></td>
<td>16(^b)</td>
<td>766 (678)</td>
</tr>
<tr>
<td>V4</td>
<td>4</td>
<td>405</td>
<td>427</td>
<td>4.4</td>
<td>1.1</td>
<td>11</td>
<td>445 (348)</td>
</tr>
<tr>
<td>V5</td>
<td>3</td>
<td>483</td>
<td>469</td>
<td>7.2</td>
<td>1.1</td>
<td>4</td>
<td>407 (323)</td>
</tr>
<tr>
<td>V6</td>
<td>4</td>
<td>288</td>
<td>261</td>
<td>4.5</td>
<td>1.5</td>
<td>23(^c)</td>
<td>253 (268)</td>
</tr>
<tr>
<td>V7</td>
<td>3</td>
<td>298</td>
<td>249</td>
<td>5.8</td>
<td>1.8</td>
<td>6</td>
<td>421 (351)</td>
</tr>
<tr>
<td>V8</td>
<td>4</td>
<td>642</td>
<td>363</td>
<td>13.2</td>
<td>1.0</td>
<td>8</td>
<td>(366(^h)) (300(^h)) (221(^h))</td>
</tr>
<tr>
<td>V9</td>
<td>4</td>
<td>586</td>
<td>476</td>
<td>7.2</td>
<td>1.2</td>
<td></td>
<td>(386(^e)) (586(^e)) (221(^e))</td>
</tr>
<tr>
<td>C1</td>
<td>3</td>
<td>1039</td>
<td>479</td>
<td>23.0</td>
<td>6.9</td>
<td>7</td>
<td>1815 (500)</td>
</tr>
<tr>
<td>C2</td>
<td>4</td>
<td>1450</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>1748 (508)</td>
</tr>
<tr>
<td>C3</td>
<td>4</td>
<td>900</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>1280 (219)</td>
</tr>
<tr>
<td>C4</td>
<td>4</td>
<td>1080</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2191 (803)</td>
</tr>
<tr>
<td>C5</td>
<td>4</td>
<td>524</td>
<td>620</td>
<td>7.5</td>
<td>2.7</td>
<td>8</td>
<td>1882 (816)</td>
</tr>
<tr>
<td>C6</td>
<td>5</td>
<td>2183</td>
<td>823</td>
<td>28.6</td>
<td>14.9</td>
<td>7</td>
<td>2505 (256)</td>
</tr>
<tr>
<td>C7</td>
<td>2</td>
<td>1188</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>1675 (487)</td>
</tr>
<tr>
<td>C8</td>
<td>3</td>
<td>1400</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>4152 (1106)</td>
</tr>
<tr>
<td>C9</td>
<td>4</td>
<td>2068</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>1724 (567)</td>
</tr>
<tr>
<td>C10</td>
<td>3</td>
<td>572</td>
<td>538</td>
<td>8.9</td>
<td>3.9</td>
<td>6</td>
<td>2031 (804)</td>
</tr>
</tbody>
</table>

\(^{a}\) Patient diagnosis was confirmed by measuring elevated plasma alloisoleucine for classic MSUD (C) and variant MSUD (V); elevated excretion of branched-chain oxo-acids in urine (C); strongly elevated leucine levels in plasma (C); leucine tolerance (C); enzyme activity in fibroblasts (C and V); and mutation analysis (C and V). The cutoff for total leucine concentration indicating a positive screening result that requires confirmation diagnosis ranged for the different screening laboratories from 275 to 393 \( \mu \text{mol/L} \). Reference values for plasma BCAA concentrations during confirmation procedure were for leucine <230, isoleucine <105, and valine <480 \( \mu \text{mol/L} \).

\(^{b}\) Values for valine (Val), total leucine/phenylalanine (Xle/Phe), and total leucine/alanine (Xle/Ala) were not always reported from the screening laboratory.

\(^{c}\) Control sample (dried blood spots (DBS)) before confirmation diagnosis by amino acid analysis.

\(^{d}\) Control sample (DBS) had normal BCAA; however, ratios to phenylalanine were still elevated. Confirmation of diagnosis was made by amino acid analysis on day 714 of life. Data excluded from statistics. Patient V8 was referred to a metabolic clinic only after multiple requests of the screening laboratory.

\(^{e}\) Control sample (DBS) had normal BCAA (in a laboratory not specialized in newborn or selective screening). Confirmation of diagnosis was made by amino acid analysis on day 268 of life. Data excluded from statistics. Patient V9 was referred to a metabolic clinic only after multiple requests of the screening laboratory.
ably discriminate between classic and variant MSUD by NBS results. In confirmation diagnosis by amino acid analysis performed on day 4–23, differentiation was unambiguous, with a mean plasma leucine concentration of 447 (161) μmol/L in variant MSUD in contrast to 2100 (791) μmol/L in classic MSUD (P = 0.001). Importantly, no newborn with variant MSUD needed emergency management.

Our data show that an unambiguous discrimination between variant and classic MSUD is impossible by NBS. Therefore the detection of any elevation of total leucine in NBS requires immediate referral to a specialized metabolic unit or a local pediatrician/physician (in consultation with a metabolic specialist) to confirm the diagnosis by quantitative determination of plasma amino acids and to institute treatment. Measurement of plasma leucine concentrations by amino acid analysis clearly differentiates between classic and variant MSUD, which is important for choosing the proper treatment. Most likely, leucine and isoleucine concentrations in early postnatal life are crucially influenced by the extent of neonatal protein catabolism, which might differ considerably between individuals, and may account for the reported missed cases of variant MSUD (3).

Oglesbee et al. (4) recently described a method to quantify the isobaric amino acids leucine, isoleucine, alloisoleucine, and hydroxyproline as a second-tier test from the initial NBS dried blood spots. Only samples with both increased total leucine and increased alloisoleucine would lead to further metabolic workup. This approach appears promising for further reducing false-positive rates. Even with this improvement, however, discrimination between classic and variant MSUD may not be possible from the NBS results (5).

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References

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Association of the FABP2 T54 Variant with Plasma Triglycerides and Insulin Resistance in a Multiethnic Population

To the Editor:

Fasting triglyceride concentrations and insulin resistance vary substantially according to patient ethnic origin (1,2), but the role of genetic variants in these differences is not known. The fatty acid binding protein 2, intestinal (FABP2) gene encodes the intestinal fatty acid binding protein, which is involved in intestinal fatty acid uptake. Carriers of the T54 variant (c.163G>A; p.A54T) of FABP2 produce a modified, functional intestinal fatty acid binding protein that has high affinity for fatty acids. Thus carriers have higher triglyceride concentrations and insulin resistance than noncarriers (3). However, many of the studies in which this association was observed did not account for variations in health behaviors (e.g., physical activity and diet) and adiposity, which may also affect triglyceride concentrations and insulin resistance. Also, among individuals who consume a high-fat diet, T54 carriers may have higher lipids than noncarriers (4). We investigated the association of the FABP2 T54 variant with fasting triglyceride concentrations and insulin resistance in a multiethnic population, and by controlling for ethnicity, health behaviors, and adiposity we specifically focused on the explanatory power of this genetic variable. We also tested for interactions of the T54 variant with dietary variables.

Study participants were selected by stratified random sampling from 3 urban centers in Canada. The study population included a total of 972 persons of 3 ethnic origins, South Asian (n = 337), Chinese (n = 313), and European (n = 322). The mean (SD) age of this population was 49.6 (9.9) years; 51.5% were women (n = 501); 9.8% (n = 95) had confirmed type 2 diabetes; and 5.6% (n = 54) reported having had a cardiovascular disease episode (heart attack, stroke, or angina). Participants completed questionnaires on medical history, tobacco use, alcohol intake, physical activity, and diet. Study participants underwent an oral glucose tolerance test with a 75-g glucose load and provided blood samples for the analysis of fasting (8 h) triglycerides, glucose, and insulin. We used restriction isotyping to genotype all participants for the FABP2 T54 variant and the homeostasis model assessment for insulin resistance (HOMA-IR) (5). Multiple linear regression was used to measure the association of the T54 variant with triglycerides and insulin resistance after adjusting for age, sex, ethnicity, health behaviors (tobacco use, alcohol intake, physical activity, total fat intake, trans fat intake, protein intake, fiber intake, and total energy), and adiposity (body mass index and waist-to-hip ratio).

The T54 variant was present in 51.0% of all participants, and these individuals had significantly higher mean triglyceride concentrations than noncarriers (1.50 vs 1.42 mmol/L; P < 0.02). More T54 carriers than noncarriers were insulin resistant, although this difference was not statistically significant (HOMA-IR = 3.84 vs 3.41, P = 0.06). After adjustment for age, sex, and ethnicity, the T54 variant was not a significant determinant of triglyceride concentration or insulin resistance (Table 1); however, after adjustment for health behaviors and adiposity measurements, the T54 variant was significantly associated with triglyceride concentrations. This association did not differ significantly in study participants who were healthy compared with those who were undergoing treatment for high lipids or diabetes. The percentage variance in triglyceride concentrations that was attributable to the T54 variant was small (0.5%) compared to the percentage variances attributable to ethnicity (1.7%), health behaviors (4.9%), and adiposity (12.6%). After multivariate adjustment of the data, we found that the T54 variant was not significantly associated with insulin resistance. We observed no significant interactions between the T54 variant and dietary factors.

T54 prevalence, mean triglyceride concentrations, and insulin resistance were significantly higher in South Asians (60.2%, triglycerides = 1.58 mmol/L, HOMA-IR = 4.60) compared to Europeans (48.4%, triglycerides = 1.40 mmol/L, HOMA-IR = 3.08) and Chinese (43.8%, triglycerides = 1.39 mmol/L, HOMA-IR = 3.15). These differences were highly significant (overall P < 0.01; contrast P < 0.01), except for the difference between Europeans and Chinese (P > 0.29). Among South Asians, T54 carriers had significantly higher triglyceride concentrations than noncarriers (1.62 vs 1.52 mmol/L, P = 0.04) and tended to have higher insulin resistance (HOMA-IR = 4.85 vs 4.23, P = 0.18). Among Europeans, T54 carriers had higher triglyceride concentrations and insulin resistance than noncarriers, but the differences were not significant (1.43 vs 1.37 mmol/L, P = 0.31);
HOMA-IR = 3.27 vs 2.91, \( P = 0.28 \). We did not observe this pattern in Chinese participants (1.39 vs 1.39 mmol/L, \( P = 0.38 \); HOMA-IR = 3.00 vs 3.27, \( P = 0.96 \)). Adjustment for health behaviors and adiposity did not alter these associations.

Our investigation of a Canadian multiethnic population demonstrated that \( FABP2 \) T54 carriers had significantly higher triglyceride concentrations than noncarriers. Triglyceride concentrations were affected less by the T54 variant than by ethnicity, health behaviors, and adiposity. We found no evidence that the T54 variant was associated with insulin resistance or dietary interactions. Although ethnicity is probably associated with several factors that affect triglyceride concentrations (e.g., \( FABP2 \) T54, health behaviors, and adiposity), adjustment of our data for ethnicity alone was insufficient to control for confounding by these factors. Despite a higher prevalence of the T54 variant in individuals of South Asian origin, the T54 variant accounted for little variation in the high triglyceride concentrations and insulin resistance found in this ethnic group.

### Table 1. Adjusted associations of the \( FABP2 \) T54 variant with mean triglycerides and mean insulin resistance.*

<table>
<thead>
<tr>
<th>T54 status</th>
<th>Triglycerides, mmol/L (95% CI)</th>
<th>HOMA-IR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncarriers</td>
<td>1.48 (1.41 to 1.55)</td>
<td>3.49 (2.98 to 3.99)</td>
</tr>
<tr>
<td>T54 carriers</td>
<td>1.53 (1.46 to 1.60)</td>
<td>3.70 (3.20 to 4.21)</td>
</tr>
<tr>
<td>Difference (( \beta ))</td>
<td>0.05 (–0.01 to 0.11) ( P = 0.12 )</td>
<td>0.22 (–0.23 to 0.67) ( P = 0.34 )</td>
</tr>
<tr>
<td>Variance explained</td>
<td>( FABP2 ) T54, 0.25%; total variance, 10.83%</td>
<td>( FABP2 ) T54, 0.09%; total variance, 8.03%</td>
</tr>
</tbody>
</table>

* Health behaviors include tobacco use (current smoker, previous smoker, reference = never), alcohol intake (1 drink/month to 5 drinks/week, >5 drinks/week, reference = never), physical activity (metabolic equivalents/day), total fat intake (g/day), trans fat intake (g/day), protein intake (g/day), fiber intake (g/day), total energy intake (kcal/day). Adiposity metrics included body mass index (BMI, kg/m²) and waist-to-hip ratio (WHR).

### Author Contributions

**All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements:** (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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**Expert Testimony:** None declared.

**Other:** All participants in this study provided written informed consent. The study was approved by the ethics committees of McMaster University, the University of Toronto and the University of Alberta. Ethics approval for DNA analysis was obtained from the University of Western Ontario Institutional Review Board. R. He-
Robert A. Hegele has spoken on the genetic determinants of dyslipidemia and received payment from those funding the events (Merck Schering, Pfizer, and Oryx) in amounts less than $10,000.

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**References**


**To the Editor:**

Monoclonal gammopathies are usually monitored with serum and/or urine protein electrophoresis (PEL) (1). In addition, quantitative immunoglobulins are often ordered for patients with large serum M-spike. For patients with monoclonal light-chain diseases, diagnosis and monitoring can be a challenge, and free light-chain (FLC) quantification in serum has become an important additional test (2). Although there have been some conflicting reports, serum FLC should not replace urine PEL for monitoring patients with a urine M-spike (3–5). The purpose of this study was to determine if measuring urine FLCs or urine total light chains (TLCs) is useful in addition to measuring urine PEL for monitoring patients, in a manner analogous to measuring quantitative immunoglobulin as a complement to measuring serum PEL.

Sequential waste urines (n = 336) were obtained from excess samples in which a monoclonal protein was detected by urine immunofixation electrophoresis (IFE). We performed PEL assays with agarose gel electrophoresis (REP, Helena Laboratories) after we increased the protein concentration in urine samples up to 200-fold to achieve a protein concentration of 20–80 g/L. IFE assays were performed with Helena reagent sets. The FLCs and TLCs were quantified on a Dade Behring BN II nephelometer, with separate antisera for κ and λ FLCs (The Binding Site) and κ and λ TLCs (Dade Behring). The limits of quantification of the urine TLC κ and λ assays are 7 and 4 mg/L, respectively, and 1 mg/L for both κ and λ FLC. The reference range for the urine TLC κ:λ ratio was based on urine samples obtained from 54 healthy adult donors. These samples had total protein >100 mg/24 h, and we excluded 1 donor because of a highly increased TLC κ:λ ratio. The reference range for the urine FLC κ:λ ratio was based on urine samples from 91 healthy adult donors, and was the central 95% range.

Urine monoclonal protein was detected with IFE in all urine samples.
The diagnostic sensitivity of urine FLC and TLC assays is compared in Table 1. The table shows the diagnostic sensitivity for positive and negative M-spike samples, as well as the quantitation comparison to M-Spike. The correlation coefficients for the urine TLC vs the M-spike were 0.95 and 0.98 for \( \kappa \) and \( \lambda \) respectively, and 0.90 and 0.98 for the FLC assays (Table 1). These differences indicate that the standardization of these 3 assays is substantially different and reinforces the need for a traceable calibrator for the FLC assay.

Urine FLC and TLC are not sensitive diagnostic tests. Urine FLC and TLC, however, do correlate with the urine PEL M-spike, with the urine TLC being in closer agreement to the PEL M-spike. The quantification of urine TLC may therefore provide a useful quality check on measurements of patient urinary M-spike values. Like serum immunoglobulin quantification, the urine TLC assays may provide redundancy in disease monitoring by M-spike measurement.

**Table 1. Diagnostic sensitivity and quantitative comparison of urine FLC and TLC assays.**

<table>
<thead>
<tr>
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<th>FLC assay*</th>
<th>TLC assayb</th>
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<tbody>
<tr>
<td>All samples (n = 336)</td>
<td>80% (75.8%, 84.3%)</td>
<td>70% (64.8%, 74.6%)</td>
</tr>
<tr>
<td>( \kappa ) Immunotype (n = 214)</td>
<td>80% (74.5%, 85.1%)</td>
<td>67% (60.3%, 72.8%)</td>
</tr>
<tr>
<td>Positive for M-spike (n = 132)</td>
<td>95% (89.5%, 97.4%)</td>
<td>93% (88.3%, 95.7%)</td>
</tr>
<tr>
<td>Negative for M-spike (n = 82)</td>
<td>57% (46.5%, 67.5%)</td>
<td>24% (18.0%, 33.1%)</td>
</tr>
<tr>
<td>( \lambda ) Immunotype (n = 122)</td>
<td>81% (73.3%, 87.1%)</td>
<td>75% (67.1%, 82.2%)</td>
</tr>
<tr>
<td>Positive for M-spike (n = 74)</td>
<td>100% (95.1%, 100%)</td>
<td>97% (90.7%, 99.3%)</td>
</tr>
<tr>
<td>Negative for M-spike (n = 48)</td>
<td>55% (38.3%, 65.5%)</td>
<td>43% (28.8%, 55.7%)</td>
</tr>
</tbody>
</table>

| Quantitation comparison to M-Spike |
|----------------|----------------|
| \( \kappa \) Immunotype (n = 132) |
| Correlation coefficient, \( R \) | 0.90 | 0.95 |
| Linear regression, slope (SE) | 0.07 (0.003) | 0.40 (0.011) |
| \( P \) | <0.0001 | <0.0001 |
| \( \lambda \) Immunotype (n = 74) |
| Correlation coefficient, \( R \) | 0.98 | 0.98 |
| Linear regression, slope (SE) | 0.15 (0.004) | 0.60 (0.015) |
| \( P \) | <0.0001 | <0.0001 |

* Reference range for urine FLC \( \kappa: \lambda \) ratio: 1–19.

* Reference range for urine TLC \( \kappa: \lambda \) ratio: 0.7–6.2

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Precision of High-Throughput Single-Nucleotide Polymorphism Genotyping with Fingernail DNA: Comparison with Blood DNA

To the Editor:

Recently, high-throughput single-nucleotide polymorphism (SNP) genotyping arrays have been used for genome-wide association study. Blood samples are commonly used for such analyses because they provide genomic DNA of high molecular weight and in high quantities. Blood sampling is often difficult, however, when patients are located a great distance from the laboratory and the sample requires careful handling for transportation. Instead, buccal swabs or urinary epithelial cells have been used as noninvasive sources of DNA (1). Fingernail clippings are also obtained noninvasively and more easily. Such samples need no special attention for transportation and can be preserved dry at room temperature for long periods; however, because fingernail clippings yield fragmented DNA that may be contaminated with certain PCR inhibitors (2), this sample type has seemed unsuitable for analyses requiring high-quality genomic DNA. In this study, we compared the precision of Affymetrix GeneChip™ array-based SNP genotyping with fingernail DNA vs blood DNA and demonstrated the usefulness of fingernail DNA in genotyping and genome copy-number analysis.

The study protocol was approved by the Ethics Committee on Human Genome and Gene Analysis, Nagasaki University, and written informed consent was obtained from every volunteer. Ten milliliters of peripheral blood and 10 fingernail clippings were obtained from 5 healthy volunteers. Blood DNA was extracted with the standard phenol-chloroform method. Clipped fingernails were frozen in liquid nitrogen and crushed into fine powder with Multibeads Shocker™ (Yasu Kikai). The nail powder was dissolved in a urea-containing lysis solution (2 mol/L urea, 5 g/L SDS, 10 mmol/L Tris-HCl, pH 7.5, 50 mmol/L EDTA) containing 1 g/L proteinase K and 40 mmol/L dithiothreitol and was incubated overnight at 55°C. Nail DNA was extracted by the phenol-chloroform method and suspended in 30 μL 1× Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0). Genome-wide SNP genotyping was performed with the GeneChip™ Human Mapping 250K Nsp Array according to the manufacturer’s assay manual (Affymetrix). SNP data were analyzed with GTYPE software (Affymetrix). This analysis is based on the dynamic modeling algorithm that is included in GTYPE. Genome copy-number analysis was performed with CNAG software (http://www.genome.umin. jp/) (3, 4).

The human nail plate includes soft and hard types of keratin. Hard keratin constitutes >80% of nail keratin (5) and contains large numbers of cysteine moieties linked by
multiple disulfide bonds that make the nail insoluble in lysis buffer. We froze fingernail clippings in liquid nitrogen, mechanically crushed them into as fine a powder as possible, and then treated them with 2 mol/L urea. With this procedure, we were able to lyse the nail proteins almost completely without denaturing the DNA. Consequently, 10 mg (2 pieces) of fingernail clippings yielded approximately 1 μg DNA, an amount ample for the GeneChip system.

We assumed in this study that all SNP calls for the blood samples were correct, and these calls were used as baseline values for comparison with the calls for nail DNA. The mean total call rate for the blood samples was 96.26%, and that of the nail samples was 94.76%. The mean concordance rates for homozygous and heterozygous SNPs were 99.81% and 98.78%, respectively (Table 1). The concordance rates for heterozygous SNPs in nail samples decreased as the total call rates decreased. Therefore, the discrepancy in the call rates between the 2 different sources of DNA is most likely due to some incorrect calls for heterozygous SNPs in the nail DNA. In other words, many cases of lower call rates in nail samples reflect erroneous calls as homozygous SNPs instead of as heterozygous SNPs; however, the call rates and concordance values for nail DNA were sufficient for genome-wide association studies. Moreover, we performed SNP genotyping with DNA from old fingernail clippings that had been preserved for >5 years at room temperature. The total call rate for old nail DNA was 95% or higher, showing that results for nail clippings preserved for a long time were equivalent to those obtained with fresh clippings.

We also used copy-number variation analysis to compare signal homogeneity for blood and nail DNA on the GeneChip. In this study, volunteer no. 5 had a duplication polymorphism in chromosome 8 that was clearly demonstrated in both sources of DNA (data not shown). The results of the present copy-number variation analysis showed that the results obtained with nail samples were equivalent to those for blood DNA with respect to the accuracy of detecting such variation.

In conclusion, our study comparing blood and fingernail DNA with respect to SNP-genotyping accuracy on the 250K Affymetrix GeneChip systems revealed that nail DNA was as useful as blood DNA for both genome-wide association studies and genome copy-number analysis. Our results reinforce the merits of using nail samples, because nails can be stored at room temperature for a long period and need not be processed immediately for DNA extraction.

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