



ELSEVIER

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Dataset for genotyping validation of cytochrome P450 2A6 whole-gene deletion (*CYP2A6*4*) by real-time polymerase chain reaction platforms

Makiko Shimizu, Tomoki Koyama, Izumi Kishimoto, Hiroshi Yamazaki *

Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan

ARTICLE INFO

Article history:

Received 22 September 2015

Received in revised form

15 October 2015

Accepted 16 October 2015

Available online 25 October 2015

Keywords:

*CYP2A6*1**CYP2A6*4*

Copy number assays

Real-time PCR

TaqMan

Whole-gene deletion

ABSTRACT

This data article contains a supplementary figure and validation data relating to the research article entitled “Genotyping of wild-type cytochrome P450 2A6 and whole-gene deletion using human blood samples and a multiplex real-time polymerase chain reaction method with dual-labeled probes” (Shimizu et al., *Clinica Chimica Acta* 441, 71–74, 2015), which presents a multiplex real-time polymerase chain reaction method with dual-labeled probes for human P450 2A6 wild-type and whole-gene deletion. Real-time methods have dramatically improved the speed of complex genetic diagnostics compared to conventional assays based on restriction enzyme digestion. Here, we show the basic assay validation data by single and multiplex determinations in comparison with commercial TaqMan copy number assays for P450 2A6.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license

(<http://creativecommons.org/licenses/by/4.0/>).

Specifications table

Subject area	Biology
More specific subject area	Human molecular genetics

DOI of original article: <http://dx.doi.org/10.1016/j.cca.2014.12.018>

* Corresponding author. Tel./fax: +81 42 721 1406.

E-mail address: hyamazak@ac.shoyaku.ac.jp (H. Yamazaki).

<http://dx.doi.org/10.1016/j.dib.2015.10.019>

2352-3409/© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Type of data	Table, gel image, text file
How data was acquired	Applied Biosystems 7300 Real-Time PCR System
Data format	Analyzed data, validated with three independent methods.
Experimental factors	Human blood drop samples and DNA fractions obtained from 45 Japanese individual subjects.
Experimental features	A rapid detection assay of wild-type and whole-gene deletion-type of human <i>P450 2A6</i> by multiplex real-time polymerase chain reaction and commercially available TaqMan assays for <i>P450 2A6</i> copy number determinations were validated.
Data source location	Showa Pharmaceutical University, Machida, Tokyo, Japan
Data accessibility	The data are supplied with this article.

Value of the data

1. A real-time polymerase chain reaction (PCR) assay for *cytochrome P450 2A6* genotyping recently developed [1] was fully validated using a conventional PCR-restriction fragment length polymorphism (RFLP) method (Fig. 1).
2. Aside from the PCR-based methods outlined in this report, commercially available TaqMan assays for copy number determinations (whole-gene deletion *P450 2A6*4* genotyping in regions of limited homology) were almost validated (Table 1).
3. Genotyping strategies for *P450 2A6* wild-type (*P450 2A6*1*) and whole-gene deletion (*P450 2A6*4*) alleles defined here could be combined with highly automated genome-wide association studies, which have limited utility for identifying whole-gene deletions.
4. The dataset that supports the genotyping validations of human *P450 2A6* wild-type and whole gene deletion are provided by single and multiplex determinations in comparison with commercial TaqMan copy number assays.

1. Data

Validation of genotyping of human wild-type *P450 2A6*1* and whole-gene deletion *P450 2A6*4* by both PCR-RFLP and real-time platforms was carried out. One of the 45 subjects (genotyped as *P450*

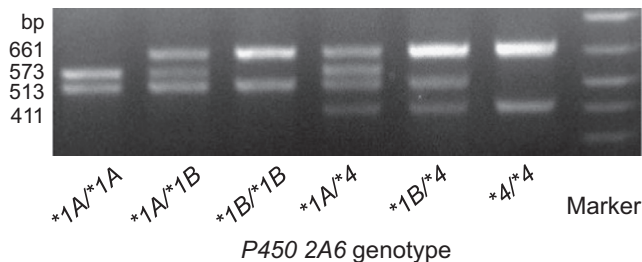


Fig. 1. A representative gel image for genotyping of human *P450 2A6* (*P450 2A6*1A*, *2A6*1B*, and *2A6*4*) by PCR-RFLP [2–4] separated on 2% agarose gel. The PCR products of *P450 2A6*1A* (1323 bp) and *2A6*1B* (1322 bp) with a forward primer named *2A6 B4* (5'-CACCGAAGTGTWCCCTATGCTG-3') and a reverse primer named *2A6 UTR AS-1* (5'-TGTAAAATGGGCATGAACGCC-3') [2–4] were digested by *FokI* into fragments of 573, 513, 148, and 89 bp and fragments of 661, 513, and 148 bp, respectively. The PCR products of *P450 2A6*4* (1309 bp) were digested by *FokI* into fragments of 661, 411, 148, and 89 bp.

Table 1

Comparison of genotyping of *P450 2A6* of 45 individuals in a Japanese population by conventional PCR (Fig. 1), multiplex real-time PCR [1], and three positions for TaqMan methods (07545274, 04488984, and 07545275).

PCR-RFLP, FokI	Multiplex real-time PCR	TaqMan			PCR-RFLP, FokI	Multiplex real-time PCR	TaqMan		
		07545274	04488984	07545275			07545274	04488984	07545275
*1A/*1A	*1/*1	1.8	1.9	2.4	*1B/*1B	*1/*1	1.8	1.9	2.2
*1A/*1A	*1/*1	2.0	1.9	2.3	*1B/*1B	*1/*1	1.7	2.1	2.4
*1A/*1A	*1/*1	1.8	2.1	2.4	*1B/*1B	*1/*1	1.9	1.8	2.4
*1A/*1A	*1/*1	2.0	2.0	2.0	*1B/*1B	*1/*1	2.2	2.0	2.5
*1A/*1A	*1/*1	2.0	2.1	2.1	*1B/*1B	*1/*1	2.1	1.7	2.1
*1A/*1A	*1/*1	2.2	2.3	2.3	*1B/*1B	*1/*1	2.2	2.5	2.1
*1A/*1B	*1/*1	1.8	1.6	1.7	*1B/*1B	*1/*1	2.5	2.8	2.3
*1A/*1B	*1/*1	1.9	2.0	2.4	*1B/*1B	*1/*1	2.1	2.1	2.2
*1A/*1B	*1/*1	2.1	2.1	2.2	*1B/*1B	*1/*1	1.7	1.8	2.2
*1A/*1B	*1/*1	2.2	1.8	2.4	*1A/*4	*1/*4	1.1	1.0	1.2
*1A/*1B	*1/*1	1.9	1.9	2.0	*1A/*4	*1/*4	1.4	1.4	1.2
*1A/*1B	*1/*1	1.9	2.0	2.4	*1A/*4	*1/*4	1.3	1.3	1.5
*1A/*1B	*1/*1	1.8	1.9	2.2	*1A/*4	*1/*4	1.0	1.2	1.0
*1A/*1B	*1/*1	1.9	2.3	2.4	*1A/*4	*1/*4	1.0	1.3	1.2
*1A/*1B	*1/*1	1.5	1.9	2.5	*1B/*4	*1/*4	1.1	1.0	1.3
*1A/*1B	*1/*1	2.1	2.0	2.5	*1B/*4	*1/*4	1.2	0.9	1.4
*1A/*1B	*1/*1	2.1	2.5	1.7	*1B/*4	*1/*4	1.2	1.3	1.2
*1A/*1B	*1/*1	2.0	2.3	2.5	*1B/*4	*1/*4	1.2	1.3	1.3
*1A/*1B	*1/*1	1.9	2.3	2.5	*1B/*4	*1/*4	1.0	1.2	1.1
*1A/*1B	*1/*1	2.0	2.0	2.0	*1B/*4	*1/*4	1.0	1.1	1.2
*1A/*1B	*1/*1	1.7	2.2	1.7	*4/*4	*4/*4	0.1	0.1	0.1
*1B/*1B	*1/*1	0.9	1.3	1.1	*4/*4	*4/*4	0.2	0.2	0.2
*1B/*1B	*1/*1	1.6	1.9	2.2					

Bold and italic copy numbers obtained by the TaqMan system were not consistent with the genotyping by conventional PCR and multiplex real-time PCR [1]. A representative PCR-RFLP gel after *FokI* digestion for *P450 2A6**1A, *1B and *4 are shown in Fig. 1.

2A6*1B/*1B, Table 1) was not matched in term of *P450 2A6* copy numbers (around 1) in the current TaqMan analysis.

2. Experimental design, materials and methods

The ethics committee of Showa Pharmaceutical University approved this analysis in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained for experimentation from all subjects. Genotyping of *P450 2A6* (*P450 2A6**1A, *2A6**1B, and *2A6**4) was carried out by conventional PCR amplification (Fig. 1) as described previously [2–4]. Blood samples were obtained with puncture needles, lysed, and stabilized [5]. Blood samples from healthy non-smoking Japanese volunteers or DNA fractions extracted separately by the standard protocol from the volunteers' buccal cells were used for real-time detection of *P450 2A6* wild-type and whole-gene deletion [1]. The samples also underwent TaqMan Copy Number Assays (Hs07545274, Hs04488984, and Hs07545275; ThermoFisher Scientific, Waltham, MA, USA). The *P450 2A6* copy numbers in the human genomes were calculated using CopyCaller software according to the manufacturer's instructions with designed human *RNase P* TaqMan Copy Number Reference Assays (4403326; ThermoFisher Scientific) after duplex real-time PCR reactions.

Conflicts of interest

None.

Acknowledgments

This work was supported in part by the Japan Society for the Promotion of Science KAKENHI Grant Number 25460198 and an SRF Grant for Biomedical Research in Japan. We thank Drs. Kazuma Kiyotani, Ren Sawaya, Shotaro Uehara, and Norie Murayama for their support of this work and David Smallbones for English language advice.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2015.10.019>.

References

- [1] M. Shimizu, R. Sawaya, I. Kishimoto, H. Yamazaki, Genotyping of wild-type cytochrome P450 2A6 and whole-gene deletion using human blood samples and a multiplex real-time polymerase chain reaction method with dual-labeled probes, *Clin. Chim. Acta* 441 (2015) 71–74.
- [2] M. Fujieda, H. Yamazaki, T. Saito, K. Kiyotani, M.A. Gyamfi, M. Sakurai, H. Dosaka-Akita, Y. Sawamura, J. Yokota, H. Kunitoh, T. Kamataki, Evaluation of *CYP2A6* genetic polymorphisms as determinants of smoking behavior and tobacco-related lung cancer risk in male Japanese smokers, *Carcinogenesis* 25 (2004) 2451–2458.
- [3] M. Shimizu, K. Kiyotani, H. Kunitoh, T. Kamataki, H. Yamazaki, Different effects of *TERT*, *TP63*, and *CYP2A6* polymorphism on individual risk of tobacco-related lung cancer in male Japanese smokers, *J. Cancer Ther.* 2 (2011) 690–696.
- [4] M. Shimizu, Y. Ishii, M. Okubo, H. Kunitoh, T. Kamataki, H. Yamazaki, Effects of *ADH1C*, *ALDH2*, and *CYP2A6* polymorphisms on individual risk of tobacco-related lung cancer in male Japanese smokers, *J. Cancer Ther.* 4 (2013) 28–35.
- [5] P.B. Nielsen, M.S. Petersen, V. Ystaas, R.V. Andersen, K.M. Hansen, V. Blaabjerg, M. Refstrup, Sample-to-SNP kit: a reliable, easy and fast tool for the detection of HFE p.H63D and p.C282Y variations associated to hereditary hemochromatosis, *Gene* 507 (2012) 79–84.