



TOHOKU
UNIVERSITY

SNP Discovery in Drug-Metabolizing Enzyme Genes and Functional Characterization of the Variant Proteins

**Masahiro Hiratsuka
Tohoku University**

Table of Contents

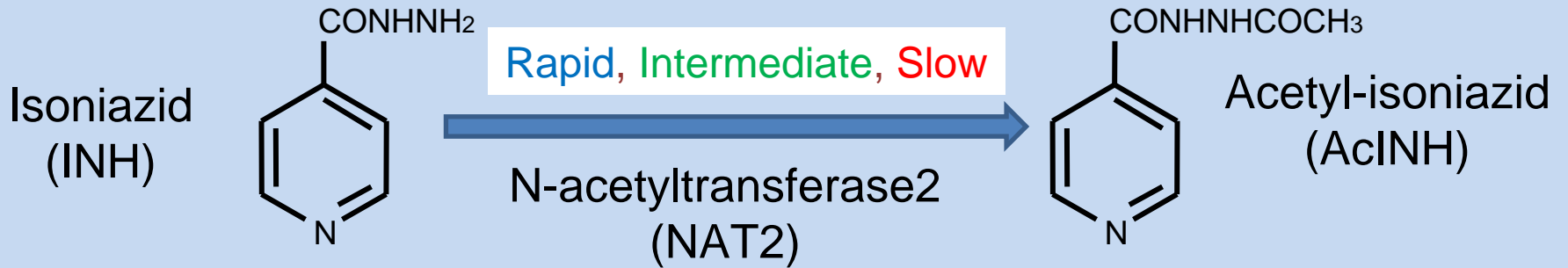
1. Background and Motivation





2. Highlight Data

- ~ Identification of novel DME SNPs
- ~ Functional analysis of CYP2D6 variants

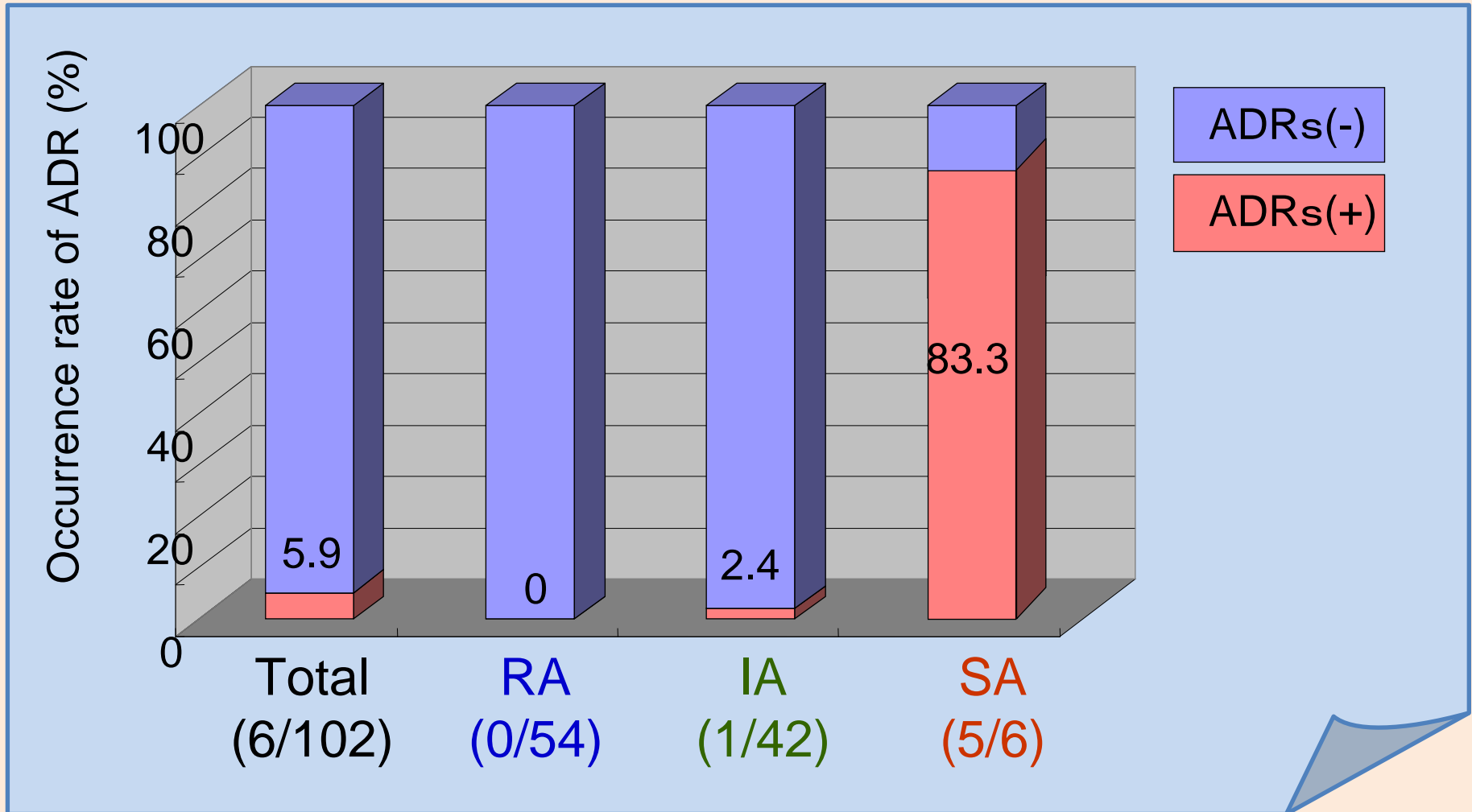
3. Summary

Background



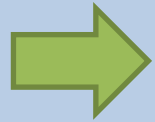
				(%)	RA
	NAT2*4 (wild-type)	*4/*4	48.4	(50%)	
		*4/*5	1.1		
	NAT2*5	*4/*6	29.7	IA (40%)	
		*4/*7	10.4		
		*5/*5	0		
	NAT2*6	*5/*6	0.5	SA (10%)	
		*5/*7	0		
	NAT2*7	*6/*6	3.1		
		*6/*7	4.2		
		*7/*7	2.6		

Incidence of Isoniazid Adverse Drug Reactions Varies with NAT2 Genotypes



Hiratsuka *et al.* DMPK, 17: 357-362 (2002)

Motivation



Investigated known SNP within DME genes...

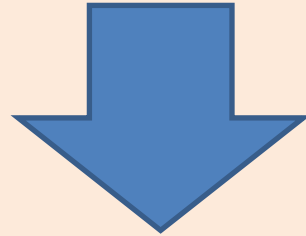
1. Identify novel SNP in DME

CYPs, TPMT, XO, NAT, DPYD....

In Japanese population

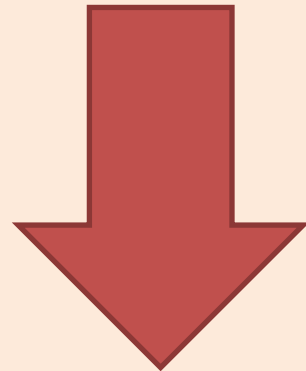
2. Evaluate the activity of the variant proteins

SNP discovery in DME genes



Direct sequencing

Expensive...



Denaturing HPLC (DHPLC)

**High sensitivity
Speed
Automation
Low-cost**

SNP discovery in DME genes using DHPLC

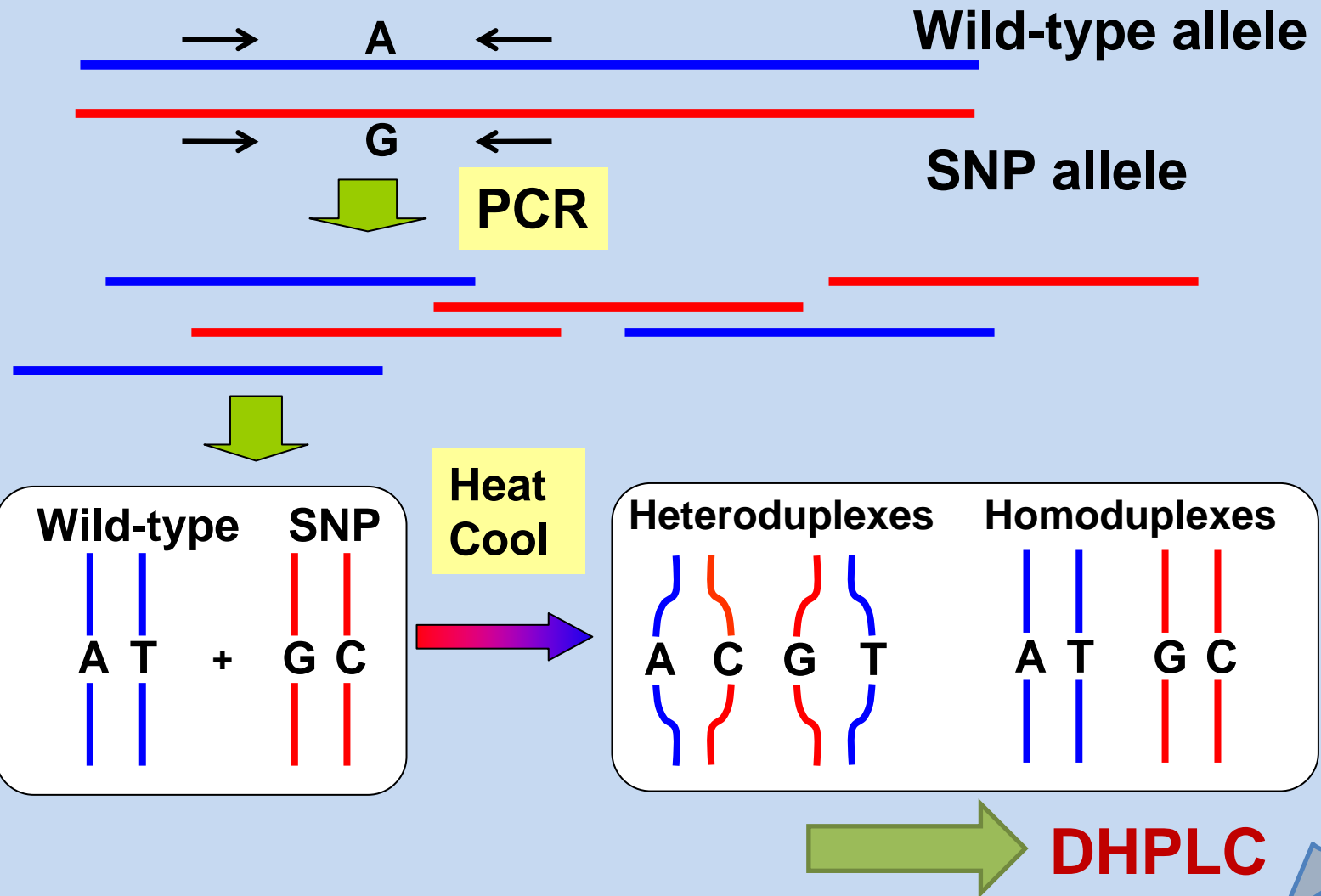
DHPLC
Transgenomic WAVE system



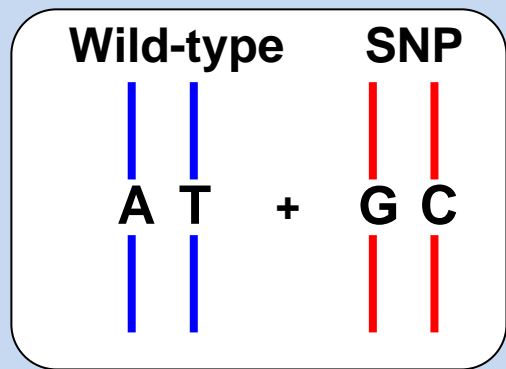
DNA separation
cartridge



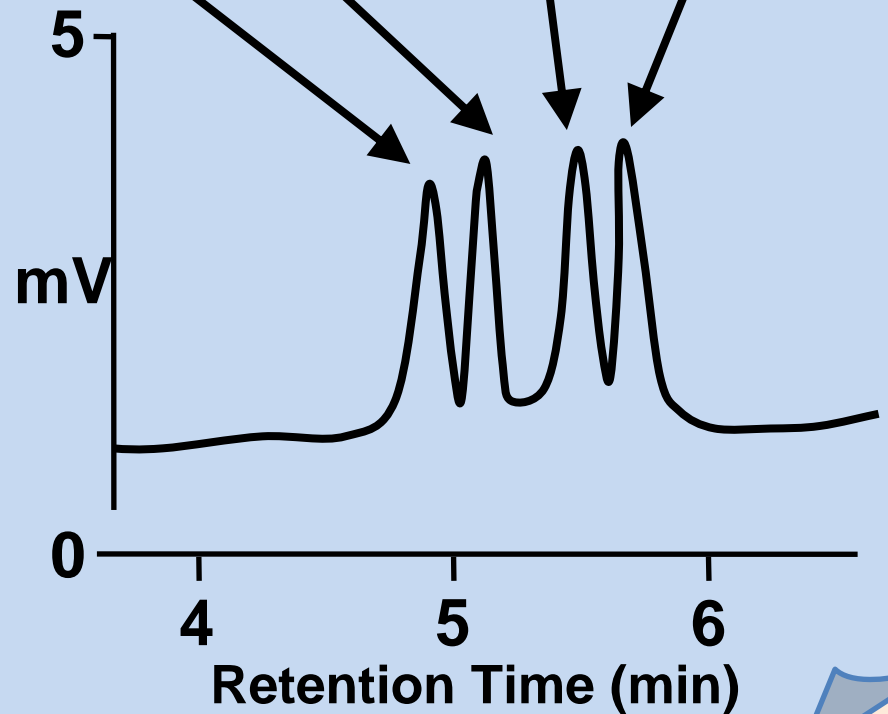
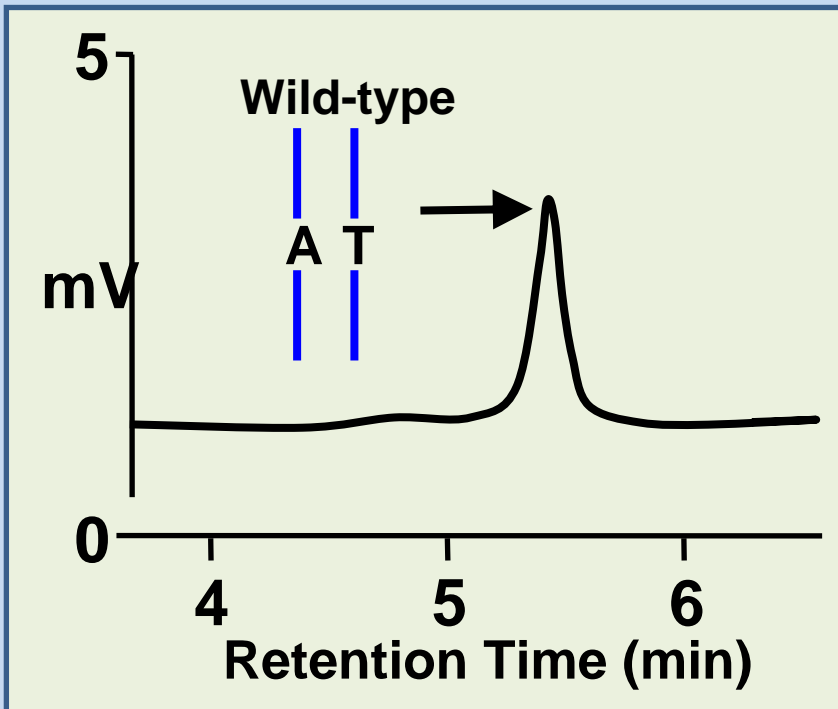
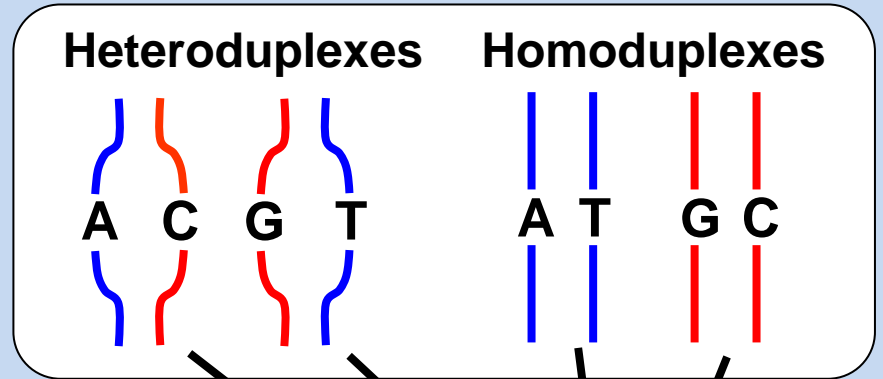
Identification of SNP by DHPLC



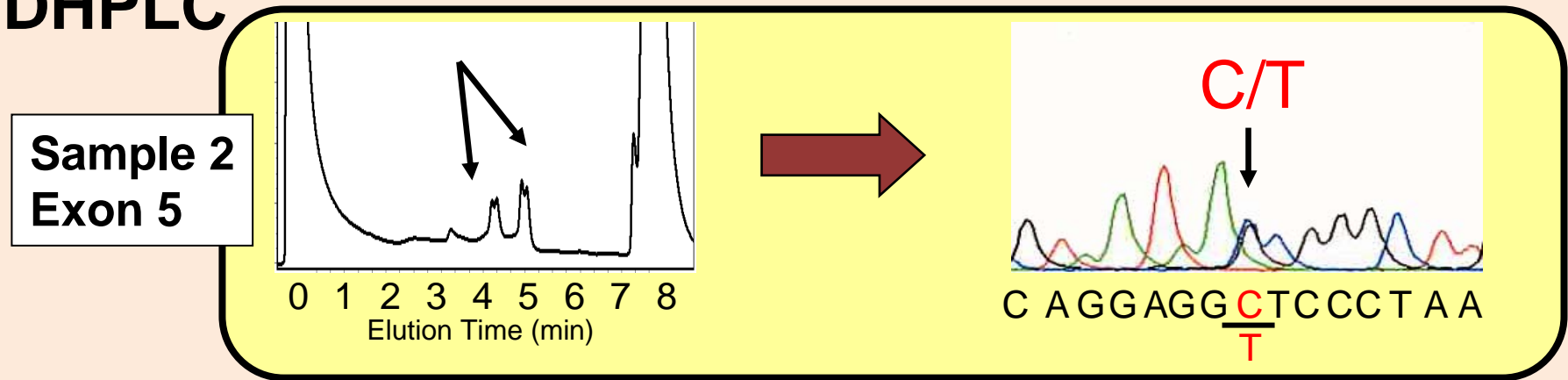
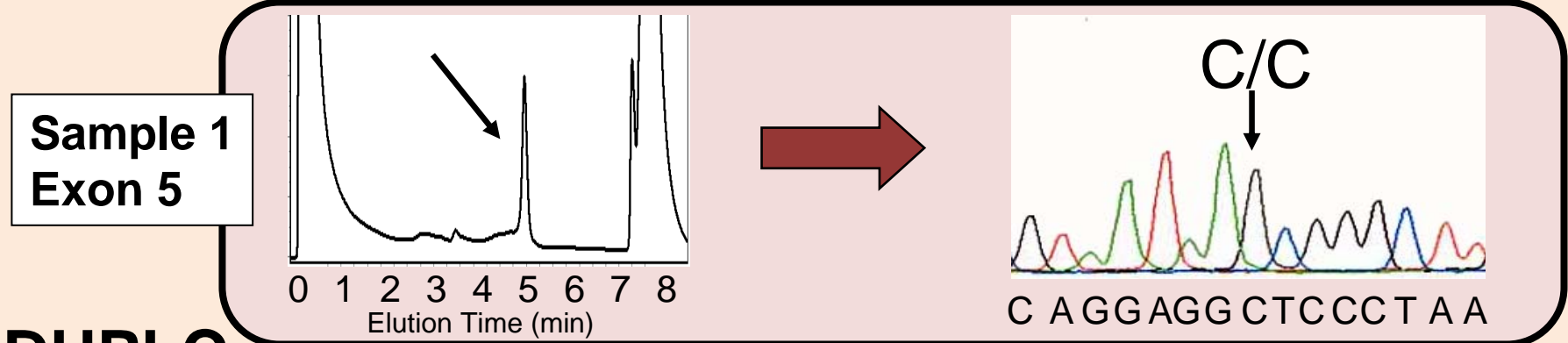
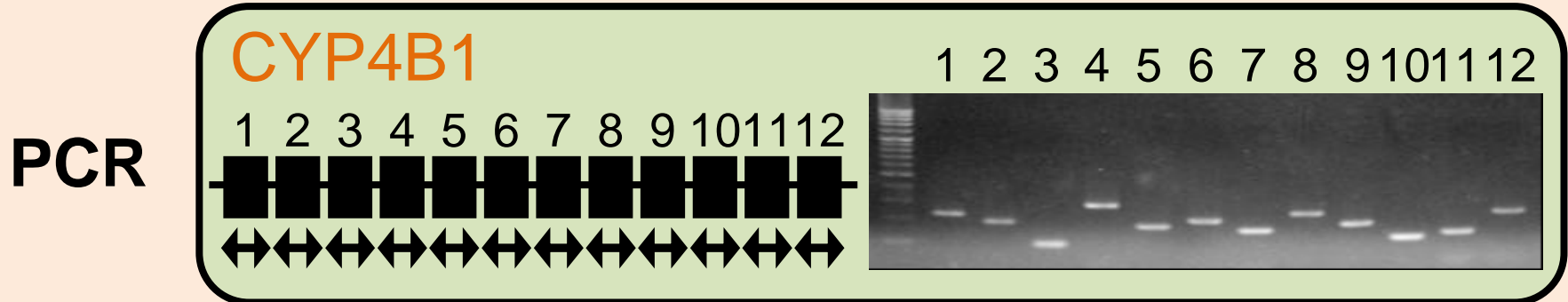
Elution profiles on DHPLC



Heat
Cool



SNP screening of CYP4B1 gene



Published Data

Drug Metab. Pharmacokinet.

CYP2B6 *23-*25 (2004), CYP4B1 *6-*7 (2004)

CYP2D6 *53-*55 (2005), MTHFR (2005)

TPMT (2006)

CYP2S1 *5A (2007)

IMPDH1, IMPDH2, HGPRT, ITPA, GMPS (2009)

XO promoter SNP (2010)

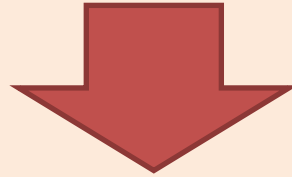
Mutat. Res.

CYP4A22 *1-*15 (2006)

Drug Metab. Dispos. Pharmacogenet. Genom.

CYP2W1 *1-*6 (2008) XO coding SNP (2008)

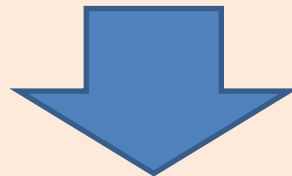
Functional Characterization of DME allelic variant proteins



cDNA expressed proteins

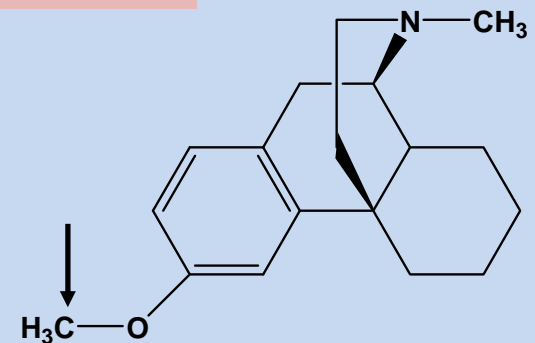
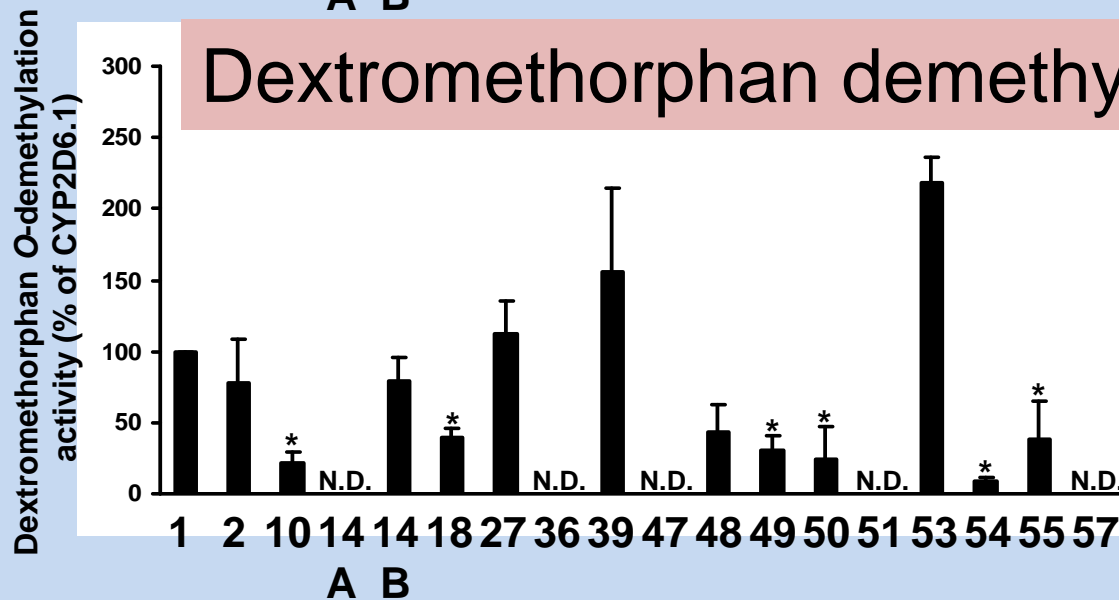
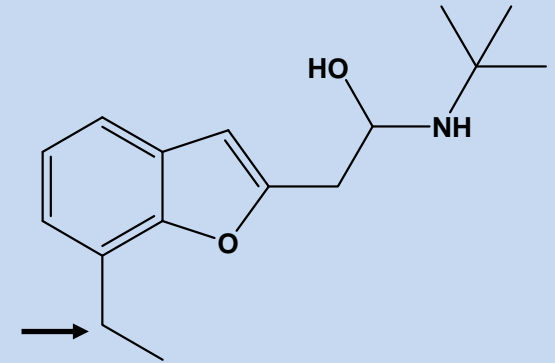
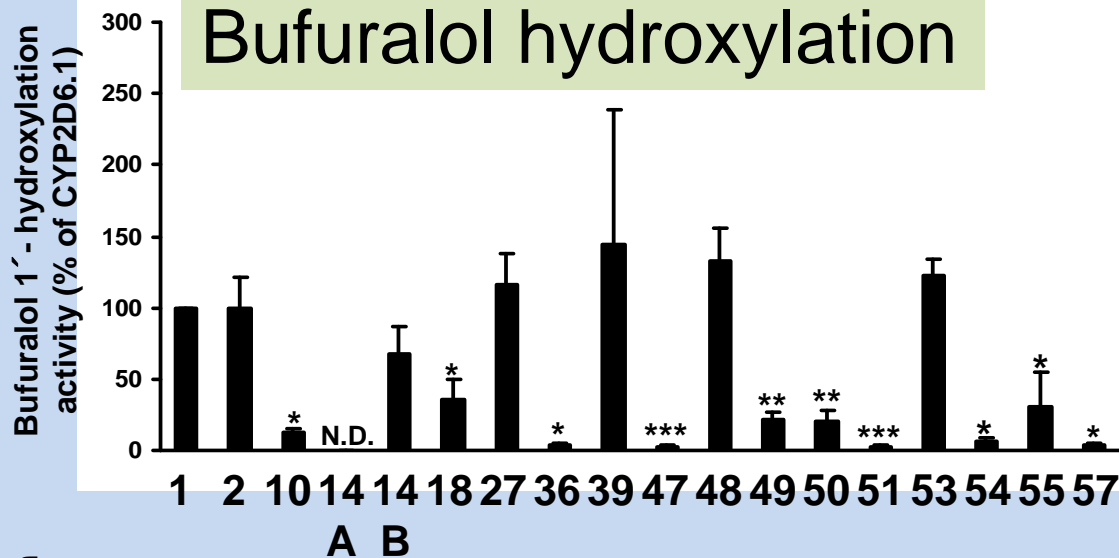


E. Coli, Insect cells, Yeast, Mammalian cells

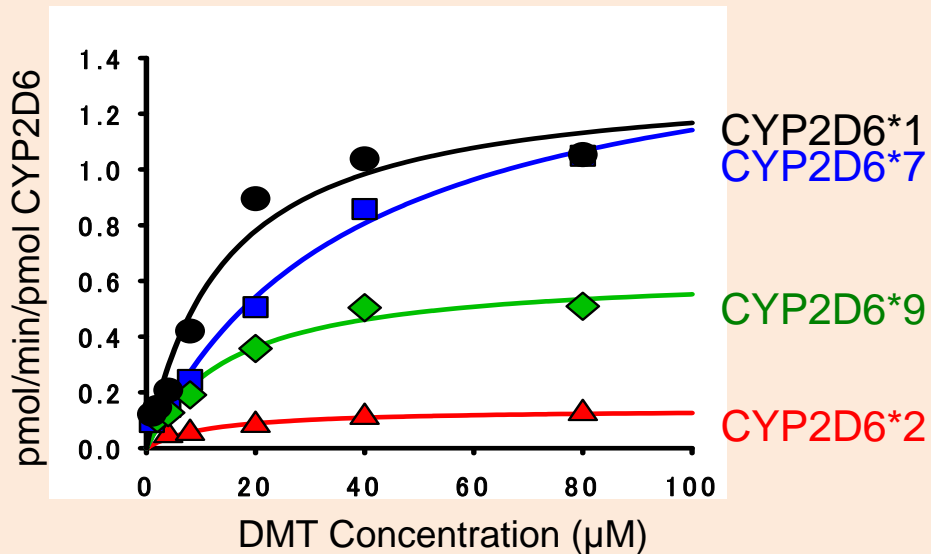
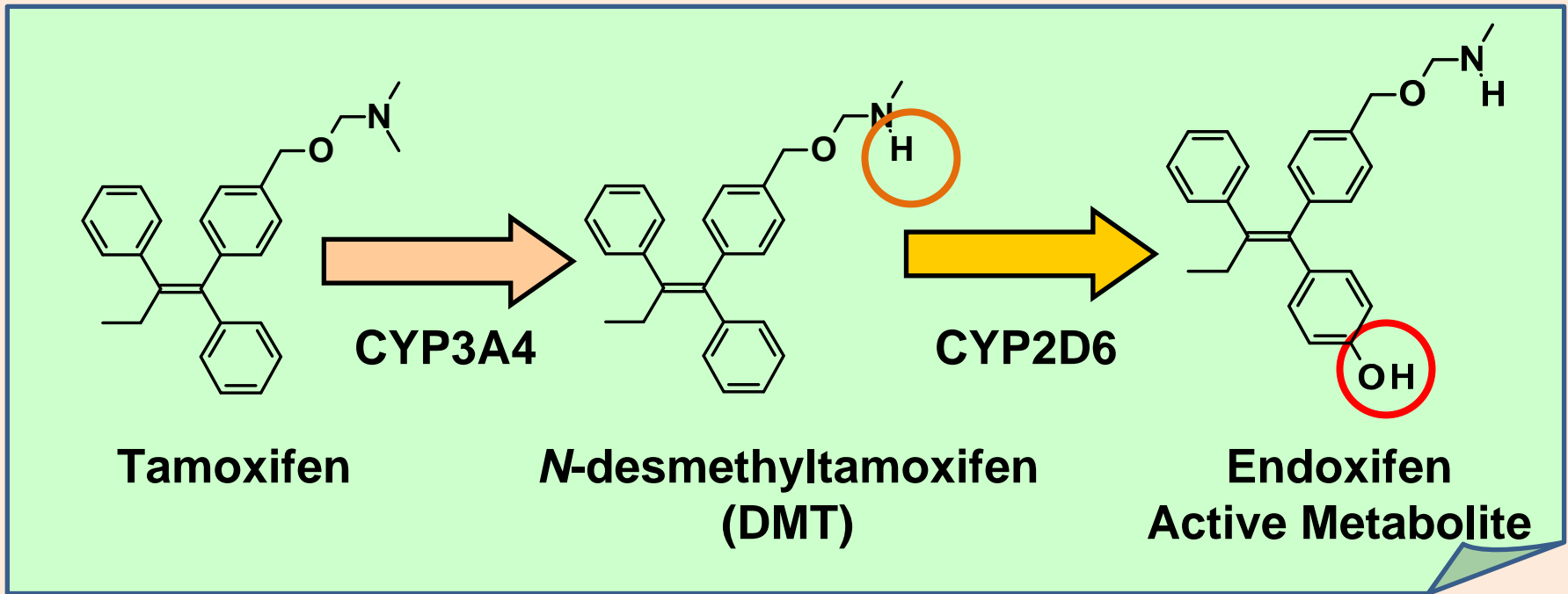


COS-7 cells

Activity of CYP2D6 variant proteins



Endoxifen generated by CYP2D6



CYP2D6*10?

Substrate-dependent alterations of CYP2D6 variants

Bufuralol hydroxylation

Variants	K_m (μM)	V_{max} (pmol/min/pmol)	CL_{int} ($\mu\text{L}/\text{min}/\text{pmol}$)
<i>CYP2D6*1</i>	7.14 ± 1.13	3.24 ± 0.97	0.47 ± 0.16
<i>CYP2D6*10</i>	11.3 ± 1.35 *	0.30 ± 0.12 **	0.03 ± 0.01 **

N-Desmethyldesmetamoxifen hydroxylation

Variants	K_m (μM)	V_{max} (pmol/min/pmol)	CL_{int} ($\mu\text{L}/\text{min}/\text{pmol}$)
<i>CYP2D6*1</i>	2.96 ± 1.29	1.03 ± 0.39	0.35 ± 0.12
<i>CYP2D6*10</i>	N.D.	N.D.	N.D.

* $P < 0.05$, ** $P < 0.01$

Published Data

Gene	The number of variant	Substrates	Journal
CYP2D6	18	Bufuralol Dextromethorphan	Drug Metab. Dispos. (2008)
XO	22	Xanthine 6-thioxanthine	Pharmacogenet. Genom. (2008) DMPK (2010)
TPMT	24	6-thioguanine	Pharmacogenet. Genom. (2008)
CYP2B6	27	Selegiline 7-EFC	Pharmacogenet. Genom. (2010)
CYP2D6 CYP2B6 CYP2C9	50 28 34	N-desmethyiltamoxifen Artemether Warfarin	Ongoing!

Summary

- **SNP discovery in DME genes**
 - ***DHPLC**
 - ***Novel SNPs in DME genes in Japanese**
- **Functional characterization of the variant proteins**
 - ***cDNA expression in COS-7 cells**
 - ***The most comprehensive**

Acknowledgement

Tohoku Pharmaceutical University

Takamitsu Sasaki, Michinao Mizugaki,
Yumiko Konno, Tomomi Saito,
Mutsumi Kudo, Kanako Sakuyama,
Hisayoshi Nozawa, Aiko Ebisawa,
Naoko Tsukamoto, Toshihiro Shida,
Yudai Hinai, Toshiko Moteki, Yoko Sasaki,
Yuya Katsumoto, Yoshiyuki Hanzawa,
Miho Horikawa, Shuta Ujiie,
Kiyoshi Nagata, Masaaki Ishikawa,
Kiminori Ota, Yasuyuki Endo,
Yorihisa Tanaka

Karolinska Institutet, Stockholm

Magnus Ingelman-Sundberg

UCSF, San Francisco

Michael Baldwin

Tohoku University

Noriyasu Hirasawa
Takanori Hishinuma
Yoichi Matsubara

Yuka Muroi (CYP2D6)
Masashi Honda (CYP2B6)
Yuichiro Tamaki
(CYP4B1)
Yui Niinuma (CYP2C9)

