Electronic Supplementary Material

Clinical Pharmacokinetics

Oral yohimbine as a new probe drug to predict CYP2D6 activity: results of a fixed sequence phase 1 trial

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Demographic data of the study participants

Supplementary Table S1. Demographic data of the study participants, Gene dose was determind according to Gaedigk et al [1].

No	Height [cm]	Weight [kg]	Age [years]	BMI [kg/m2]	Gender	Geno-type	*CYP2D6* Alleles	Gene dose
1	165	64	24	19.8	w	PM	*4/*4	0
2	163	50	29	18.8	w	EM	*1/*2	2
5	190	91	38	25.2	m	EM	*1/*4	1
6	192	110	44	29.8	m	IM	*4/*9	0.5
7	190	87	25	24.1	m	IM	*4/*10	0.5
8	173	74	91	24.7	m	EM	*1/*4	1
9	165	58	29	21.3	w	EM	*2/*2	2
10	181	82	21	25.0	m	PM	*4/*5	0
11	187	75	33	23.7	m	EM	*1/*35	2
12	181	71	23	21.7	w	EM	*1/*1	2
13	181	63	32	19.2	m	EM	*1/*1	2
14	166	68	59	24.7	w	EM	*1/*2	2
15	185	82	28	24.0	m	PM	*3/*4	0
16	168	88	55	31.2	w	EM	*2/*41	2
17	159	52	24	20.6	w	EM	*1/*1	2
18	182	85	27	25.7	m	PM	*4/*5	0

CYP2D6 genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

CYP2D6 genotyping was performed using a modification of the single-base primer extension method by Sistonen *et al*. [1]. First, the CYP2D6 gene was selectively amplified using the ExpandTM Long Template PCR System (Roche Diagnostics GmbH, Mannheim, Germany) and primers CYP2D6-F_long and CYP2D6-R_long (Supplementary Table S1). The 18 µl reaction mixture contained 3 µl genomic DNA (about 150 ng), 1X Expand Long Buffer 1, 0.7 mM $MgCl_2$, 0.32 mM dNTPs, 0.18 µM of each primer, and 1.25 U Expand Long DNA Polymerase Mix. The PCR reaction was carried out at 94 °C for 2 min, 30 cycles of 96 °C for 10 s and 68 °C for 4 min 20 s, and a final elongation at 68 °C for 7 min. The PCR product was purified using 5 U rShrimp Alkaline Phosphatase and 4 U exonuclease I (both Thermo Fisher Scientific, Darmstadt, Germany) and incubation at 37 °C for 1 h, followed by heat-inactivation at 80 °C for 15 min.

Second, the single-base primer extension reaction was carried out using the SNaPshotTM Multiplex Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Briefly, 1X SNaPshot Multiplex Ready Reaction Mix and 1X CYP2D6 SNaPshot Primer Mix (primer sequences and concentrations are listed in Supplementary Table S2) were mixed with 2 µl purified PCR product and the reaction was carried out for 25 cycles at 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 30 s. The PCR product was purified using 0.5 U FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific) and incubation at 37 °C for 1 h, followed by heat-inactivation at 75 °C for 15 min.

Third, 1 µl of purified reaction product was mixed with 9 µl Hi-DiTM Formamide and 0.2 µl GeneScanTM 120 LIZTM dye Size Standard, heated to 95 °C for 5 min, and analyzed on the 3500xL Genetic Analyzer capillary electrophoresis system using POP-7TM Polymer and a 36 cm 24-Capillary Array (all Thermo Fisher Scientific). Genotypes were called using the GeneMapperTM Software 6.0, enabling the identification of CYP2D6 alleles **1-4*, **6*, **9*, **10*, **17*, **35*, and **41*.

Whole gene duplication or deletion of CYP2D6 was identified by long PCR using primers P13_new, P24_new, and P81_new for the deletion allele (*CYP2D6*5*) and primers CYP-17 and CYP-32 for the whole gene duplication (*CYP2D6*MxN*), respectively, modified from Sistonen *et al*. [1]. For both PCRs, 16 µl reaction mixture contained 1 µl genomic DNA (about 50 ng), 1X Expand Long Buffer 1, 0.77 mM $MgCl_2$, 0.35 mM dNTPs, 0.19 µM of each primer, and 0.75 U Expand Long DNA Polymerase Mix. The PCR reactions were carried out at 94 °C for 2 min, 35 cycles of 96 °C for 10 s, 70 °C (deletion) or 57 °C (duplication) for 20 s, and

68 °C for 5 min, and a final elongation at 68 °C for 7 min. Ten µl of PCR product were analyzed on a 0.8 % agarose gel. For the deletion PCR, fragment sizes were 3.5 kb for the *CYP2D6*5* deletion allele and 4.5 kb for non-deleted alleles. For the duplication PCR, fragment sizes were 3.6 kb for duplicated CYP2D6 alleles (*CYP2D6*MxN*) and 4.5 kb for non-duplicated alleles.

In case of whole gene duplication of CYP2D6, the duplicated allele was identified using a modification of the single-base primer extension method described above. First, duplicated CYP2D6 alleles were selectively amplified using the ExpandTM Long Template PCR System and primers Lx2F_new1 and Lx2R_new1 (Supplementary Table S1). The 27 µl reaction mixture contained 2 µl genomic DNA (about 100 ng), 1X Expand Long Buffer 1, 0.74 mM $MgCl_2$, 0.33 mM dNTPs, 0.18 µM of each primer, and 1.5 U Expand Long DNA Polymerase Mix. The PCR reaction was carried out at 94 °C for 2 min, 35 cycles of 96 °C for 10 s, 61 °C for 20 s, and 68 °C for 7 min, and a final elongation at 68 °C for 7 min. The PCR product was purified as described above. Second, the single-base primer extension reaction was carried out using the SNaPshotTM Multiplex Kit as described above but with a different primer pool (primer sequences and concentrations are listed in Table 1). The reaction products were purified and analyzed on the 3500xL Genetic Analyzer as described above, enabling the identification of duplicated CYP2D6 alleles **1*, **2*, and **35* (fully active), **10* and **41* (reduced activity), and **4* (inactive).

Supplementary Table S2. Sequences and concentrations of the primers used for CYP2D6 genotyping

Name	**Sequence 5'->3'**	**Concentration in 10X primer mix** [µM]
SNaPshot pre-PCR		
CYP2D6-F_long	ATCCCAGAAGGCTTTGCAGGCTTCA	
CYP2D6-R_long	CCGACTGAGCCCTGGGAGGTAGGTA	
SNaPshot PCR		
2850C>T	gatcgatCAGGTCAGCCACCACTATGC	0.25
1846G>A	gatcgatcgatcgatcTACCCGCATCTCCCACCCCCA	0.25
1707T>del	gatcgatcgatcgatcgatcgGGCAAGAAGTCGCTGGAGCAG	0.5
2613-15GA>del	gatcgatcgatcgatcgatcgatcgaGCCTTCCTGGCAGAGATGGAG	0.5
100C>T	gatcgatcgatcgatcgatcgatcgatcgatCAACGCTGGGCTGCACGCTAC	1.5
4180G>C	gatcgatcgatcgatcgatcgatcgatcgatcgatcgCAAAGCTCATAGGGGGATGGG	1.5
1023C>T	gatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgaCCGCCCGCCTGTGCCCATCA	1.5
2988G>A	gatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgAACAGTGCAGGGGCCGAGGGAG	1.0
31G>A	gatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatCCAGGAGCAGGAAGATGGCCACTATCA	2.5
2549A>del_var3	gatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgaGGGCTGGGTCCCAGGTCATCC	5.0
Deletion PCR		

P13_new	CCCACACCAGGCACCTGTACTCCTCA	
P24_new	ACAGGCATGAGCTAAGGCACCCAGACTA	
P81_new	GCCCCCGTCTAGTGGGGAGACAAAC	
Duplication PCR		
CYP-17	TCCCCCACTGACCCAACTCT	
CYP-32	CACGTGCAGGGCACCTAGAT	
Duplication SNaPshot pre-PCR		
Lx2F_new1	CGGCCCAGCCACCATGGTGTCTTTGCTTTC	
Lx2R_new1	CGACACCGGATTCCAGCTGGGAAATG	
Duplication SNaPshot PCR		
-1584G>C	gatcgatcgCCTGGACAACTTGGAAGAACC	2
100C>T	gatcgatcgatcgatcgatcgatcgatCAACGCTGGGCTGCACGCTAC	3
4180G>C	gatcgatcgatcgatcgatcgatcgatcgatcgCAAAGCTCATAGGGGGATGGG	3.5
31G>A	gatcgatcgatcgatcgatcgatcgatcgatcgatcgatCCAGGAGCAGGAAGATGGCCACTATCA	2.5

References

1. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther. 2008 Feb;83(2):234-42.

2. Sistonen J, Fuselli S, Levo A, Sajantila A. CYP2D6 genotyping by a multiplex primer extension reaction. Clin Chem. 2005 Jul;51(7):1291-5.