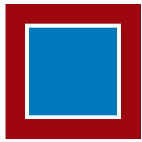
THE PRIMARY CARE COMPANION FOR CNS DISORDERS

Supplementary Material

Article Title: Genetic and Clinical Factors Affecting Plasma Clozapine Concentration

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Supplementary Table 1. Primer Sequences for Amplification of Polymerase Chain Reaction Fragments That Contained the Polymorphism, Polymerase Chain Reaction Fragment Size, and Restriction Endonucleases Used in Polymerase Chain Reaction-Restriction Fragment Length Polymorphism*

Gene and Single Nucleotide Polymorphism	Primer (5´-3´)	Fragment Length (bp)	Restriction Enzyme	Reference
UGT1A4 -142T>G	fo-GTTGGGCCCATAACGAAAGGCAGTT	576	FastDigest StuI (Eco1471)	[18]
rs2011425	re-GCTCCACACACAACACCTATGAAG			

*DNA fragments spanning the single nucleotide polymorphism were amplified in individual reactions by polymerase chain reaction (10 ng DNA/reaction) containing the forward and reverse primers. The temperature program was 95°C for 5 min, followed by 50 cycles of 95°C for 30 s, 55.5°C for 1 min, and 72°C for 1 min. The amplified 576-bp fragment was digested (FastDigest StuI, Eco1471, Fischer Scientific, Stockholm, Sweden) for 10 min at 37°C and analyzed on 1% agarose gels. The wild type allele, and not the mutant allele, was cut into 324- and 252-bp fragments.

Supplementary Table 2. Assay Identification and Sequences of Interest For Genotype Determination*

Gene and Single Nucleotide Polymorphism	Assay Identification	Sequence of Interest
CYP1A2 -729C>T rs12720461	C__30634146_10	GGCTAGGTGTAGGGGTCCTGAGTTC[C/T]GGGCTTTGCTACCCAGCTCTTGACT
CYP1A2 -163C>A rs762551	C___8881221_40	TGCTCAAAGGGTGAGCTCTGTGGGC[C/A]CAGGACGCATGGTAGATGGAGCTTA
CYP1A2 -2467delT rs35694136	C__60142977_10	TGCAGTGAGCCATGATTGTGGCACA[T/-]GAACCCCAACCTGGGTGACAGAGCA
MDR1 -3435C>T rs4005995	C___7586657_20	TGTTGGCCTCCTTTGCTGCCCTCAC[A/G]ATCTCTTCCTGTGACACCACCCGGC

*Primers and probes were obtained commercially (Life Technologies, Stockholm, Sweden). DNA fragments spanning the single nucleotide polymorphisms were amplified in individual reactions by polymerase chain reaction (10 ng DNA/reaction) containing forward primer, reverse primer, and probes for the single nucleotide polymorphisms. Polymerase chain reaction was performed in 96- or 384-well formats with 2 negative controls distributed per assay. The temperature program was 95°C for 10 min followed by 50 cycles of 92°C for 15 s and 60°C for 90 s.

Supplementary Table 3. Polymerase Chain Reaction Primers, Sequencing Primers, and Dispensing Order Used For MDR1 -2677G>T Genotype Determination*

Gene and Single Nucleotide Polymorphism	**Primer (5´-3´)**	**Dispensing Order**
MDR1 -142T>G rs2032582	foB-CTGGACAAGCACTGAAAGATAAGA re-TGGCTTTGCTACTTTCTGTAAGTT seq-TTAGTTTGACTCACCTTCC	GCAGCTAGCT

*Forward, reverse, and sequencing primers were obtained commercially (Fischer Scientific, Stockholm, Sweden). DNA fragments spanning the single nucleotide polymorphism were amplified in individual reactions by polymerase chain reaction (10 ng DNA/reaction) containing the forward and reverse primers for the single nucleotide polymorphism. The temperature program was 95°C for 10 min followed by 50 cycles of 92°C for 15 s and 58.5°C for 90 s. The amplified DNA fragments spanning the single nucleotide polymorphism were analyzed with a sequencer (PSQ96, QIAGEN Nordic, Solna, Sweden).

Supplementary Table 4. Genotype Frequencies With Corresponding Concentration-To-Dose Ratios of Clozapine*

Gene and Single Nucleotide Polymorphism	Genotype	No. of Patients (%)	Concentration-To-Dose Ratios
CYP1A2			
rs35694136	TT	86 (91)	5 ± 4
CYP1A2*1D -2467delT	T/del	7 (7)	5 ± 3
	del/del	2 (2)	2.7 ± 0.5
rs12720461	CC	95 (100)	5 ± 4
CYP1A2*K -729C>T	CT	0 (0)	
	TT	0 (0)	
rs762551	CC	9 (9)	4 ± 2
CYP1A2*F -163C>A	CA	35 (37)	6 ± 4
	AA	51 (54)	4 ± 2
MDR1			
rs1045642	TT	32 (34)	5 ± 3
-3435C>T	CT	49 (52)	5 ± 4
	CC	14 (15)	5 ± 4
rs2032582	TT	13 (14)	5 ± 3
-2677G>T	GT	51 (54)	6 ± 4
	GG	26 (27)	4 ± 2
	TA	2 (2)	4 ± 2
	GA	3 (3)	8 ± 8
	AA	0 (0)	
UGT1A4			
rs2011425	TT	84 (88)	5 ± 4
L48V -142T>G	TG	10 (11)	5 ± 3
	GG	1 (1)	4.1

*Data reported as mean ± SD.