CYP2D6: clozapine 1529/1530/1531

AUC = area under the concentration-time curve, CO = clozapine-N-oxide, Css = steady state concentration, DC = N-desmethylclozapine, IM = intermediate metaboliser (gene dose 0.25-1) (decreased CYP2D6 enzyme activity), NM = normal metaboliser (gene dose 1.25-2.5) (normal CYP2D6 enzyme activity, NS = non-significant, PANSS = Positive and Negative Syndrome Scale, PM = poor metaboliser (gene dose 0) (absent CYP2D6 enzyme activity), RR = relative risk, S = significant, UM = ultra-rapid metaboliser (gene dose ≥ 2.75) (increased CYP2D6 enzyme activity)

Brief summary and justification of choices:
Clozapine is primarily metabolised by CYP1A2, to a limited extent by CYP3A4 and possibly by CYP2C19 and CYP-2C9.
None of the studies showed a relationship between CYP2D6 genotype and clozapine response or the occurrence of side effects. However, Lesche 2020 did show a small decrease in clozapine exposure with increasing genotype-predicted CYP2D6 activity after correction for co-medication with CYP2D6 inhibitors. This decrease was too small to affect clozapine response. For this reason, the KNMP Pharmacogenetics Working Group concluded that there is a gene-drug interaction, but that therapy adjustment is not required for the variant phenotypes (yes/no interactions).
An overview of the clinical and kinetic effects per phenotype is provided in the background information text of the gene-drug interactions in the KNMP Kennisbank. You may also have access to this background text via your pharmacy or physician electronic decision support system.

The table below uses the KNMP nomenclature for NM, PM, IM and UM. As a result, the definitions of NM, PM, IM and UM in the table below can differ from the definitions used by the authors in the article.

<table>
<tr><th>Source</th><th>Code</th><th>Effect</th><th>Comments</th></tr>
<tr>
<td>ref. 1
Ortega-Vázquez A et al. Alcohol intake potentiates clozapine adverse effects associated to CYP1A2*1C in patients with refractory psychosis. Drug Dev Res 2020 Dec 17 (online ahead of print). PMID: 33336447.</td>
<td>3</td>
<td>48 patients were treated with clozapine (10-700 mg/day, mean 189 mg/day).
Steady-state plasma concentrations were determined. Adverse drug reactions were determined after 18 weeks of clozapine treatment and were classified into neurological, metabolic and general adverse drug reactions.
Relevant co-medication was not excluded.
Analysis of adverse events was performed with multiple linear regression analysis correcting for sex, sedentary lifestyle, smoking status alcohol intake, and CYP1A2- and CYP2C19-genotypes, and with correction of P-values for multiple comparisons using the Benjamini-Hochberg procedure.

Genotyping:
<table>
<tr><th></th><th></th><th colspan="3">number of patients</th></tr>
<tr><th>gene variant</th><th>alleles</th><th>no gene variant</th><th>heterozygous</th><th>homozygous</th></tr>
<tr><td>100C>T</td><td>*10, *4</td><td>35</td><td>4</td><td>9</td></tr>
<tr><td>1847G>A</td><td>*4</td><td>38</td><td>10</td><td></td></tr>
<tr><td>*5</td><td>*5</td><td>47</td><td>1</td><td></td></tr>
<tr><td>1022C>T</td><td>*17, *40</td><td>47</td><td></td><td>1</td></tr>
<tr><td>gene duplication</td><td>gene duplication</td><td>46</td><td>1</td><td>1</td></tr>
</table>
Results:
<table>
<tr><td colspan="4">Dose- and weight-corrected plasma concentration of clozapine compared to no gene variant:</td></tr>
<tr><td>gene variant (alleles)</td><td>homozygous</td><td>heterozygous</td><td>value for no gene variant</td></tr>
</table>
</td>
<td>Authors' conclusion:
'Overall, CYP variants showed no effect on clozapine pharmacokinetics.''</td>
</tr>
</table>

<table>
<tr>
<td>ref. 1, continuation</td>
<td>IM: AA

IM or PM: AA
UM: AA</td>
<td>
<table>
<tr><td>*10, *4</td><td>x 1.07
NS for homozygous versus heterozygous versus no gene variant</td><td>x 0.52</td><td>64.088 ng/ml per mg/kg</td></tr>
<tr><td>*4</td><td></td><td>x 0.80 (NS)</td><td>65.083 ng/ml per mg/kg</td></tr>
<tr><td>*5</td><td></td><td>x 0.55</td><td>63.011 ng/ml per mg/kg</td></tr>
<tr><td>*17, *40</td><td>x 0.92</td><td></td><td>62.529 ng/ml per mg/kg</td></tr>
<tr><td>gene duplication</td><td>x 0.66</td><td>x 12.51</td><td>60.934 ng/ml per mg/kg</td></tr>
<tr><td colspan="4">There was also no significant effect on the metabolic ratio clozapine/N-desmethylclozapine for any of the investigated gene variants (NS).</td></tr>
</table>

<table>
<tr><td colspan="5">Adverse events:</td></tr>
<tr><td>comparison</td><td>total adverse events</td><td>general adverse events</td><td>neurological adverse events</td><td>metabolic adverse events</td></tr>
<tr><td>heterozygous+homozygous *10 or *4 compared to no *10 or *4</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>heterozygous *4 compared to no *4</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>homozygous *17 or *40 compared to no *17 or *40</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>homozygous *10 or *4 compared to no+heterozygous *10 or *4</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="5">Note: the authors claim that the effect on neurological adverse events for homozygous *10 or *4 compared to no+heterozygous *10 or *4 becomes significant after adjusting the P-value for the False Discovery Rate. However, because adjusting for False Discovery Rate should diminish the number of significant results and not increase it, this result does not seem to be valid and is not included in the table.</td></tr>
</table>

NOTE: Genotyping was also performed for *3, but this variant was not found in this patient population.
The determined gene variants are the most important gene variants in this Mexican population.
</td>
<td></td>
</tr>
<tr>
<td>ref. 2
Lesche D et al. Impact of CYP-1A2, CYP2C19, and CYP2D6 genotype- and phenoconversion-predicted enzyme activity on clozapine exposure and symptom severity. Pharmacogenomics J 2020;20:192-201.
PubMed PMID: 31616047.</td>
<td>4</td>
<td>66 patients were treated with clozapine (mean 408 mg/day).
The total score from the Positive and Negative Syndrome Scale (PANSS) was used to assess schizophrenia symptom severity.
Relevant co-medication was not excluded, but CYP2D6 activity scores were corrected for known inducers (smoking) and inhibitors. In the presence of a strong CYP2D6 inhibitor (fluoxetine), the CYP2D6 activity score was multiplied by 0. In the presence of a moderate CYP2D6 inhibitors (escitalopram/citalopram and sertraline), the CYP2D6 activity score was multiplied by 0.5. 47% of patients was smoker, 8% used a strong CYP2D6 inhibitor and 20% used a moderate CYP2D6 inhibitor.
Clozapine plasma concentrations were adjusted for quantile–quantile (Q–Q) plots. P-values were corrected for multiple testing.

Genotyping:
- 41x NM
- 20x IM
- 5x PM
Genotype- and co-medication-predicted phenotypes were: 35x NM, 22x IM and 9x PM.</td>
<td>Authors' conclusion:
''These findings highlight the clinical importance of nongenetic factors (smoking, concomitant medications) and suggest that the added utility of CYP1A2, CYP-2D6, and CYP-2C19 activity scores to guide clozapine dosing is currently limited.''</td>
</tr>
</table>

<table>
<tr>
<td>ref. 2, continuation</td>
<td>IM: A
PM: A</td>
<td>Results:
<table>
<tr><td colspan="3">Associations of genotype-predicted and genotype- and co-medication-predicted CYP2D6 activity:</td></tr>
<tr><td rowspan="3">dose-corrected clozapine trough concentration</td><td>genotype-predicted</td><td>trend for a decrease with increasing CYP2D6 activity (p = 0.106) (NS)</td></tr>
<tr><td rowspan="3">genotype-and co-medication predicted</td><td>S for a decrease with increasing CYP2D6 activity</td></tr>
<tr><td>Genotype- and co-medication-predicted CYP2D6 activity explained 7% of the variation in clozapine trough concentration.</td></tr>
<tr><td>In a model together with genotype- and smoking-predicted CYP1A2 activity, genotype- and co-medication-predicted CYP2D6 activity explained an additional 2% of the variation in clozapine trough concentration.</td></tr>
<tr><td rowspan="2">symptom severity</td><td>genotype-predicted</td><td>NS</td></tr>
<tr><td>genotype-and co-medication predicted</td><td>NS</td></tr>
</table>
NOTE: Genotyping was performed for *2-*10, *14, *17, *36, *41, *114 and gene duplication. These are the most important gene variants in this Australian population.</td>
<td></td>
</tr>
<tr>
<td>ref. 3
Tóth K et al. Potential role of patients' CYP-3A-status in clozapine pharmacokinetics. Int J Neuropsychopharmacol 2017;20:529-37.
PubMed PMID: 28340122.</td>
<td>3

IM+PM: AA
UM: AA</td>
<td>92 patients were treated with clozapine.
Relevant co-medication was not excluded.

Genotyping:
- 36x NM (gene dose 2)
- 37x IM+NM (gene dose 1/0 + gene dose 1.25-1.5)
- 14x IM+PM (gene dose 0.5/0,5 or gene dose 0.25-0.5 + PM)
- 5x UM

Results:
<table>
<tr><td colspan="2">Result for IM+PM versus IM+NM versus NM (gene dose 2) versus UM:</td></tr>
<tr><td>dose- and bodyweight-corrected clozapine trough concentration</td><td>NS</td></tr>
</table>
NOTE: Genotyping was performed for *3-*6, *10, *41 and gene duplication. These are the most important gene variants in this Hungarian population.</td>
<td>Authors’ conclusion:
‘’The patients’ CYP2C19 or CYP2D6 genotypes and CYP-1A2 expression seemed to have no effect on clozapine serum concentration’’</td>
</tr>
<tr>
<td>ref. 4
Akamine Y et al. Quantification of the steady-state plasma concentrations of clozapine and N-desmethylclozapine in Japanese patients with schizophrenia using a novel HPLC method and the effects of CYPs and ABC transpor-</td>
<td>3

IM+PM: AA</td>
<td>41 patients were treated with a fixed clozapine dose of 100-600 mg/day for at least four weeks.
Relevant co-mediation was not excluded.

Genotyping:
- 11x NM (gene dose 2)
- 18x NM+IM (gene dose 1-1.5)
- 12x IM+PM (11x gene dose 0-0.5, 1x PM)

Results:
<table>
<tr><td></td><td>IM+PM</td><td>NM+IM</td><td>value for NM (gene dose 2)</td></tr>
<tr><td>median dose-corrected clozapine trough concentration</td><td>x 1.15 (NS)</td><td>x 1.27 (NS)</td><td>1.40 ng/ml.mg</td></tr>
</table></td>
<td>Authors’ conclusion:
‘’There were no significant differrences in trough concentration/ dose ratios of clozapine and N-desmethylclozapine among ABCB1, CYP2D6 or CYP3A5 genotypes.’’</td>
</tr>
</table>

ters polymorphisms. Ann Clin Biochem 2017;54:677-85. PubMed PMID: 27932669.		median dose-corrected N-desmethylclozapine trough concentration	x 1.31 (NS)	x 1.51 (NS)	0.45 ng/ml.mg	
		median N-desmethylclozapine/clozapine ratio	x 1.11 (NS)	x 1.03 (NS)	0.37	
		NOTE: Genotyping was performed for *2, *5 and *10. These are the most important gene variants in this Japanese population.				
ref. 5 Xu Q et al. Association studies of genomic variants with treatment response to risperidone, clozapine, quetiapine and chlorpromazine in the Chinese Han population. Pharmacogenomics J 2016;16:357-65. PubMed PMID: 26282453.	4 IM: AA PM: AA	240 patients were treated with clozapine for 2 months (initial dose 50 mg/day, gradually increased to 200-400 mg/day in week 1, adjustment guided by tolerance from week 3). Relevant co-medication was excluded. Good response was defined as a decrease in the score on the Positive and Negative Syndrome Scale (PANSS) by at least 50%. Results: Variant allele versus *1: - No difference in the percentage of patients with good response for *2, *4 and *10 (all three NS) The same result was found for all three variant alleles for *1/*1 versus (*1/variant allele) versus (variant allele/variant allele) (all three NS). NOTE: Alleles *2, *4 and *10 were genotyped. These are the genetic variants with a frequency of at least 1% in this Chinese population. The two polymorphisms of *2 were analysed separately.				
ref. 6 Lee ST et al. Association study of 27 annotated genes for clozapine pharmacogenetics: validation of preexisting studies and identification of a new candidate gene, ABCB1, for treatment response. J Clin Psychopharmacol 2012;32:441-8. PubMed PMID: 22722500.	3 IM+PM: AA	96 patients were treated with clozapine for at least 6 months. The mean dose was 319 mg/day. Patients on multiple antipsychotic drugs and users of CYP1A2 inhibitors were excluded. Co-medication was not known. There were 19 smokers. Corrections were made for dose and smoking. Clinical response did not correlate significantly with serum concentration. - None of the CYP2D6 polymorphisms were associated with dose and weight-corrected clozapine and norclozapine plasma concentrations (NS). (*4, *5, *10 and *41) versus (not *4, *5, *10 or *41): - Increase of the dose and weight-corrected clozapine concentration by 20% (from 131.8 to 158.8 ng.kg/mL per mg) (NS) - Increase of the dose and weight-corrected norclozapine concentration by 3% (from 76 to 78.3 ng.kg/mL per mg) (NS) - Increase of the dose-corrected norclozapine + clozapine concentration by 14% (from 207.8 to 237.1 ng.kg/mL per mg) (NS) NOTE: genotyping was performed for *2 to *6, *9, *10, *29, *41 and rs59421388. Gene duplication was not found in this group. The two polymorphisms of *2 were analysed separately.	Authors' conclusion: 'Among the pharmacokinetic-related single nucleotide polymorphisms, rs2069521 and rs2069522 in CYP1A2 for clozapine (dose/weight) and norclozapine (dose/weight) and rs1135840 in CYP2D6 for norclozapine/clozapine showed borderline associations that were insignificant after correction for multiple testing.'
ref. 7 Jaquenoud Sirot E et al. ABCB1 and cytochrome P450 polymorphisms: clinical pharmacogenetics of clozapine. J Clin Psychopharmacol 2009;29:319-	3	75 patients were treated with clozapine. The median clozapine dose was 250 mg/day (25-800 mg/day). No changes in co-medication were made from at least 2 weeks before the start of the study (4 weeks for fluoxetine). Co-medication other than fluvoxamine did not influence the outcome measures. Corrections were made for fluvoxamine by determining the significance for both the overall population and the fluvoxamine group. Smoking (n=45) decreased the norclozapine plasma concentration, but not that of clozapine. Genotyping: - 41x NM (40x *1/*1 and 1x *4/gene duplication) - 24x IM (4x *1/*3, 16x *1/*4, 3x *1/*5 and 1x *1/*6) - 4x PM (*4/*4)	Authors' conclusion: 'In addition, ABCB1, but not CYP2B6, CYP2C9, CYP2D6, CYP3A5, nor CYP3A7 polymorphisms, influence clozapine pharmacokinetics.'

26. PubMed PMID: 19593168. **ref. 7, continuation**	IM+PM +UM: AA	- 4x UM (*1/gene duplication) Result: CYP2D6 polymorphism did not influence clozapine, norclozapine or clozapine + norclozapine plasma concentrations. NOTE: genotyping was performed for *3 to *6 and gene duplication.	
ref. 8 Melkersson KI et al. Impact of CYP-1A2 and CYP-2D6 polymorphisms on drug metabolism and on insulin and lipid elevations and insulin resistance in clozapine-treated patients. J Clin Psychiatry 2007;68:697-704.	4 IM+PM: AA	17 patients, 1x PM, 5x IM, 11x NM, clozapine 100-600 mg/day, 6 smokers, no relevant co-medication. (IM + PM) versus NM: - No differences in C/D ratios for clozapine and N-desmethylclozapine and in the clozapine/N-desmethylclozapine Css ratio. There were no significant differences in clozapine C/D ratio between smokers and non-smokers. NOTE: genotyping was performed for *3 to *6 (the most important null alleles in the Caucasian population) and for gene duplication.	Authors' conclusion: 'Clozapine and N-desmethylclozapine C/D ratios were not related to the *CYP2D6* genotype.'
ref. 9 Dettling M et al. Long-term therapeutic drug monitoring of clozapine and metabolites in psychiatric in- and outpatients. Psychopharmacology 2000;152:80-86.	4 PM: AA IM: AA UM: AA	34 patients, 1x PM, 8x IM (1 active allele), 22x NM (2 active alleles), 1x UM (three active alleles), screened for alleles *1x2, *2x2, *2 to *12, *4x2, *14 and *17 of which *1, *2, *9, *10 and *17 are active, clozapine dosing started at 25 mg/day, guided by effect to mean 320 mg/day, no co-medication, stratified to smokers and non-smokers; - PM: clozapine C_{ss}[a] increased from 0.8 to 1.0 ng/mL/mg versus NM (NS by 25%). - IM: clozapine C_{ss}[a] **decreased** from 0.8 to 0.6 ng/mL/mg versus NM (NS by 25%). - UM: clozapine C_{ss}[a] decreased from 0.8 to 0.6 ng/mL/mg versus NM (NS by 25%). No correlation was found between clozapine response and genotype.	Clozapine Css versus NM: PM: 125% IM: 75% UM: 75%
ref. 10 Dettling M et al. Clozapine-induced agranulocytosis and hereditary polymorphisms of clozapine metabolizing enzymes: no association with myeloperoxidase and cytochrome P450-2D6. Pharmacopsychiatry 2000;33:218-20.	3 PM: AA UM: AA	108 patients including 31 with clozapine-induced agranulocytosis (1x PM, 8x IM, 21x NM, 1x UM (3 active alleles)) and 77 controls (4x PM, 22x IM, 48x NM, 3x UM (3 active alleles)), screened for alleles *3 to *6, *8 and *14, *1 = active allele, clozapine 220-260 mg/day, no co-medication; PM and UM distribution between patients with agranulocytosis and controls was equal. No relationship between genotype and development of agranulocytosis was found. NOTE: only 1 PM and 1 UM in the cases. NOTE: agranulocytosis is a multifactorial process.	
ref. 11 Arranz MJ et al. Cytochrome P4502D6 genotype does not	3 PM: AA	123 patients, 8x PM (1x *3/*3, 7x *4/*4), 115x NM + IM (3x wt/*3, 35x wt/*4, 77x wt/wt), clozapine 125-600 mg/day, co-medication and smoking not known; No correlation was found between clozapine response and genotype.	Authors' conclusion: '…and no correlation between CYP2D6 alleles

determine response to clozapine. Br J Clin Pharmacol 1995;39:417-20.			and response to clozapine was found.'
ref. 12 Dahl ML et al. Disposition of clozapine in man: lack of association with debrisoquine and S-mephenytoin hydroxylation polymorphisms. Br J Clin Pharmacol 1994;37:71-4.	3 PM: AA	15 healthy subjects, 5x PM and 10x NM# (phenotyped using debrisoquine), 10 mg single clozapine dose, no co-medication and 1 smoker; - PM: the AUC decreased from 943 to 785 nM·h versus NM# (NS by 17%) NOTE: genotype not known. Phenotyping cannot adequately distinguish between NM and IM. NM# is therefore equal to NM + IM. NOTE: the dose was very low, especially for healthy subjects.	Authors' conclusion: 'Although the power of the present study is low, our results suggest that the overall pharmacokinetics of clozapine *in vivo* are not influenced significantly by polymorphic variation in CYP-2D6 hydroxylase activities.' Clozapine AUC versus NM + IM: PM: 120%
ref. 13 SmPC Leponex (clozapine) 14-08-19.	0	Interactions: Some of the other serotonin reuptake inhibitors such as fluoxetine, paroxetine, and, to a lesser degree, sertraline, are CYP2D6 inhibitors and, as a consequence, major pharmacokinetic interactions with clozapine are less likely. Pharmacokinetics: Leponex is almost completely metabolised before excretion by CYP1A2 and CYP3A4, and to some extent by CYP2C19 and CYP2D6.	
ref. 14 SmPC Clozaril (clozapine) 11-02-21, USA.	0 PM: A	Dose/use in specific populations: Dose reduction may be necessary in patients who are CYP2D6 poor metabolizers. Clozapine concentrations may be increased in these patients, because clozapine is almost completely metabolized and then excreted. Pharmacokinetics: Clozapine is a substrate for many cytochrome P450 isozymes, in particular CYP1A2, CYP2D6, and CYP3A4. A subset (3%–10%) of the population has reduced activity of CYP2D6 (CYP2D6 poor metabolizers). These individuals may develop higher than expected plasma concentrations of clozapine when given usual doses.	

[a] Corrected for dose

Risk group	--

Comments:

- The article of Milosavljevic 2021 (Milosavljevic F et al. Association of CYP2C19 and CYP2D6 poor and intermediate metabolizer status with antidepressant and antipsychotic exposure: a systematic review and meta-analysis. JAMA Psychiatry 2021;78:270-80) was not included, because it does not contain meta-analyses for CYP2D6 and clozapine (only 2 studies for IM (Lesche 2019 and Amakine 2017) and 1 for PM (Lesche 2019) included in the analyses).
  The case report of Mian 2019 (Mian P et al. High levels of several antipsychotics and antidepressants due to a pharmacogenetic cause: a case report. Pharmacogenomics 2019;20:567-70. PMID: 31190622) describing a IM patient with high clozapine plasma concentrations was not included, because the patient concomitantly used venlafaxine at the time of the plasma concentration measurements. Venlafaxine is known to increase the clozapine plasma concentration. For this reason, it is not certain whether the high clozapine plasma concentrations were caused by the CYP2D6 IM phenotype.

Date of literature search: 27 July 2021.

	Phenotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	PM	4 A	Yes	No	13 September 2021
	IM	4 A	Yes	No	
	UM	4 AA	Yes	No	

Mechanism:
Clozapine is primarily converted by CYP1A2 to the active metabolite N-desmethylclozapine (norclozapine). Clozapine is also metabolised to a limited extent by CYP3A4 and possibly by CYP2D6, CYP2C19 and CYP2C9.