CYP1A2: clozapine 4640 to 4645

CI = confidence interval, BMI = body-mass index, HDL = high-density lipoprotein, IM = intermediate metaboliser (a fully functional or *1F allele in combination with an allele resulting in an enzyme with reduced or absent activity other than *1C) (reduced CYP1A2 enzyme activity), LDL = low-density lipoprotein, NM = normal metaboliser (two fully functional alleles) (normal CYP1A2 enzyme activity), NS = non-significant, OR = odds ratio, OR_{corr} = corrected odds ratio, PM = poor metaboliser (two alleles resulting in an enzyme with reduced or absent activity that are not both *1C) (strongly reduced or absent CYP1A2 enzyme activity), S = significant, TDM = therapeutic drug monitoring, *1A = a fully functional allele, *1C = the most common allele in the Netherlands reported to result in an enzyme with decreased activity, *1C-heterozygote = a genotype with one *1C and one other allele or genotype group *1C-heterozygote (defined as all combinations of *1C and a fully active or *1F allele, for example *1A/*1C, *1C/*1D and *1C/*1F) (reported to have reduced CYP1A2 enzyme activity), *1F = an allele reported to result in increased inducibility of CYP1A2 expression, '*1F' = an allele that contains other gene variants alongside the gene variant in *1F (-163C>A), e.g. the alleles *1K and *1W (the presence of other gene variants alongside the one in *1F has been reported to abolish the increased inducibility of CYP1A2 expression, with *1K even being reported to result in an enzyme with reduced activity), *1A/*1F = genotype *1A/*1F or genotype group *1A/*1F (defined as all combinations of an *1F allele and a fully functional allele, for example *1A/*1F, *1B/*1F and *1D/*1F), *1F/*1F = the most common genotype in the Netherlands (reported to result in increased inducibility of CYP1A2 expression).

Brief summary and justification of choices:

Clozapine is mainly converted by CYP1A2 to the active metabolite N-desmethylclozapine (norclozapine). The activity of norclozapine is low and not considered clinically relevant. Polymorphisms that influence the activity of CYP1A2 are therefore expected to influence the clozapine plasma concentrations and in turn the incidence of side effects and the effectiveness.

*1F is the most common gene variant in the Netherlands. Pharmacogenetic guidelines for *1F/*1F are therefore not useful. No abnormal activity has been demonstrated for *1D, *1W and rs2069522. Therefore, they fall under NM for the time being, as does *1A/*1A. One genome-wide association study (GWAS) showed rs2472297, an intergenic variant between CYP1A1 and CYP1A2, to be associated with reduction of clozapine plasma concentrations adjusted for, amongst others, dose (Pardiñas 2019). However, this was not confirmed by another study. In addition, PharmGKB considers rs2472297 to be a CYP1A1 variant, and an association in a GWAS does not indicate causality. For these reasons, there is not enough evidence for an effect of rs2472297 on the activity of CYP1A2, and the polymorphism was not included as a CYP1A2 variant yet.

NM and *1A/*1F *Clinical differences*

Five studies investigating *1A/*1F and/or NM found significant clinical differences versus *1F/*1F. However, none of these clinical differences were confirmed in any other articles and corresponding changes in plasma concentrations were only found in one study.

Viikki 2014 (185 patients) found a decrease in the severity of side effects for (NM + *1A/*1F). However, there were no differences in plasma concentrations of clozapine, norclozapine and clozapine + norclozapine between (NM + *1A/*1F) and *1F/*1F. In addition, Ortega-Vázquez 2020 (48 patients) did not find an effect of *1F on total adverse events, general adverse events, metabolic adverse events and neurological adverse events, Rajkumar 2013 (101 patients) did not find an effect on adverse events, and Ferrari 2012 (12 cases and 22 control patients) did not find an effect on severe adverse events.

Olsson 2015 (95 patients) found an increased risk of fasting glucose concentrations > 5.6 mmol/l for (NM + *1A/*1F). This was supported by an increase in dose-corrected clozapine concentration in this group. However, Looman 2013 (70 patients) did not find an increase in the incidence of uncontrolled glucose for NM, and Vasudev 2017 (60 patients) did not find an effect of *1F on fasting glucose levels. Moreover, Olsson 2015 reported that this may have been a coincidental finding due to multiple testing. Vasudev 2017 (60 patients) found a decreased risk of metabolic syndrome in *1F/*1F, which would correspond to an increased risk in NM+*1A/*1F, but clozapine plasma concentrations did not differ in patients with the *1F/*1F genotype in this study. A decreased risk of metabolic syndrome in *1F/*1F was only found for non-smokers in this study. In smokers with *1F/*1F there was an increased metabolic syndrome risk. There was no effect of *1F on other metabolic outcomes (BMI, fasting glucose levels, and lipid levels). Looman 2013 (70 patients) did not find an effect of *1F

on metabolic syndrome, but found NM patients to have higher plasma concentrations of HDL cholesterol than (*1A/*1F + *1F/*1F). The presence of an effect was not confirmed by analyses of clozapine plasma concentrations. In addition, Vasudev 2017 did not find an effect of *1F on lipid levels (including HDL cholesterol levels) and Ortega-Vázquez 2020 did not find an effect on metabolic adverse events. Basile 2001 (70 patients) did not find on effect of *1A on the increase in body weight during treatment for 6 weeks. It is also very likely that metabolic side effects are not dose related. The affinity for metabolic receptors seems to be high to the extent that side effects also occur at low doses and plasma concentrations. An association with kinetic genes therefore does not seem likely.

Kohlrausch 2013 found significantly fewer epileptic seizures in (*1A/*1F + NM + *1C-heterozygote + *1C/*1C), but this was not supported by a significant difference in distribution of the *1F/*1F, *1A/*1F and *1A/*1A genotype groups over the patients with and without seizures. This is also not confirmed by similar findings in other articles or supported by analyses of plasma concentrations.

De Brito 2015 found a 2-fold higher incidence of *1F/*1F in 27 super-refractory patients (treatment-resistant patients not responding to clozapine) compared to 27 refractory patients (treatment-resistant patients responding to clozapine). However, whereas the baseline Brief Psychiatric Rating Scale (BPRS) score was higher in the super-refractory patients, there was no significant difference in the BPRS score change compared to refractory patients. This raises the question whether this result really points to a difference in effectiveness of clozapine or to a difference in disease severity in these patients. In addition, Lesche 2020 (66 patients) and Rajkumar 2013 (101 treatment-resistant patients) did not find an effect of *1F on clozapine effectiveness (based on symptom severity in Lesche 2020 and on percentage of non-responders, schizophrenia symptoms, disability and cognitive function in Rajkumar 2013).

Kinetic differences

Ten kinetic studies, ranging in size from 48 to 185 patients, and one meta-analysis of 4 studies compared NM, *1A/*1F or (NM + *1A/*1F) to *1F/*1F. Eight of these studies and the meta-analysis found no significant differences (Ortega-Vázquez 2020 (48 patients), Lesche 2020 (66 patients), Na Takuathung 2019 (meta-analyses of 4 studies), Huang 2016 (143 patients), Viikki 2014 (185 patients), Lee 2012 (96 patients), Jaquenoud Sirot 2009 (75 patients), Kootstra-Ros 2005 (58 patients), van der Weide 2003 (80 patients)). Lesche 2020 showed an effect of genotype- and smoking-predicted CYP1A2 activity, however genotype and smoking together predicted less of the variance in clozapine exposure than smoking alone. Two studies with 51 and 95 patients found significantly higher plasma concentrations for NM or for (NM + *1A/*1F) (Ammar 2021 (51 patients), Olsson 2015 (95 patients)). In Ammar 2021 smoking was associated with dose-corrected clozapine trough concentration in univariate analysis, but not in multivariate analysis. So, smoking was not an independent predictor of dose-corrected clozapine trough concentration in this study. This is strange considering the well demonstrated impact of smoking on clozapine plasma concentration.

Conclusion

There is insufficient evidence for clinical or kinetic differences between NM, *1A/*1F and *1F/*1F. The KNMP Pharmacogenetics Working Group has therefore decided that there is no gene-drug interaction for NM and for *1A/*1F (no/no-interactions).

*1C/*1C and *1C-heterozygotes

Clinical differences

Ortega-Vázquez 2020 (21 heterozygotes and 7 homozygotes for the *1C-polymorphism) found an increased risk for total adverse events for heterozygotes + homozygotes for the *1C-polymorphism compared to patients without *1C-polymorphism, but no difference in dose- and weight-corrected plasma concentration between the genotypes. In addition, this was not confirmed by an increased risk for any of the three groups of adverse events (general, metabolic and neurological). In addition, other studies including more than 10 heterozygotes found no significant effect of *1C on side effects (Kohlrausch 2013 (17x *1C-heterozygotes, 1x *1C/*1C; investigating seizures only), Rajkumar 2013 (18x *1C-heterozygotes, 2x *1C/*1C), Ferrari 2012 (12x *1C-heterozygotes, 1x *1C/*1C). Only in a case-control study were all three patients with side effects found to be either *1C/*1C (n=1) or *1C/*1A (n=2), whilst the 5 control patients were all *1F/*1F (Bolla 2011). This result was supported by a significant reduction in CYP1A2 mRNA levels in lymphocytes in the *1C group compared to the *1F/*1F group. However, a study including 12 *1C-heterozygotes and 1 *1C/*1C found no effect of *1C on CYP1A2 mRNA levels in lymphocytes (Ferrari 2012).

Rajkumar 2013 (18x *1C-heterozygotes, 2x *1C/*1C) did not find an effect of *1C on clozapine effectiveness (percentage of non-responders, schizophrenia symptoms, disability, and cognitive function).

Kinetic differences

Ammar 2021 (5 *1C-heterozygotes) and Ortega-Vázquez 2020 (21 heterozygotes and 7 homozygotes for the *1C-polymorphism) found no effect of *1C on the dose-

corrected clozapine plasma concentration.
Conclusion
The KNMP Pharmacogenetics Working Group decided that there is insufficient evidence for clinical or kinetic differences between *1C/*1C, *1C-heterozygotes and *1F/*1F and therefore for a gene-drug interaction (no/no-interactions).

IM and PM There was only one case for IM (*1A/*7), with no comparison to similar patients with the *1F/*1F or *1A/*1A genotype (Allorge D et al. Identification of a novel splice-site mutation in the CYP1A2 gene. Br J Clin Pharmacol 2003;56:341-4. PubMed PMID: 12919186). Literature for PM was lacking. There is therefore insufficient evidence to support a gene-drug interaction for IM and PM (no/no-interactions).

You can find an overview of the observed clinical and kinetic effects per genotype group in the background information text of the gene-drug interactions in the KNMP Kennisbank. You may also have access to this background information text via your pharmacy or physician electronic decision support system.

The table below uses the KNMP nomenclature for CYP1A2 polymorphisms. As a result, the nomenclature in the table below can differ from the nomenclature 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
Ammar H et al. Clinical and genetic influencing factors on clozapine pharmacokinetics in Tunisian schizophrenic patients. Pharmacogenomics J 2021 Mar 17 (online ahead of print). PMID: 33731885.</td>
<td>4

*1F/*1F: A
*1A/*1F: AA
*1A/*1A: A

*1C-heterozygote: AA</td>
<td>51 patients were treated with clozapine 200-700 mg/day. Therapeutic drug monitoring was performed by determining steady state morning trough plasma concentrations.
Co-medication influencing pharmacokinetics or pharmacological effect of clozapine was excluded. 51% of patients was smoker.
Variables with p < 0.20 in univariate analysis were included in multivariate analysis (stepwise regression analysis). Smoking was one of these variables, so multivariate analysis adjusted for smoking.

Genotyping:
*1F:
- 20x *1F/*1F
- 20x *1F-heterozygous
- 11x no *1F

*1C:
- 46x no *1C
- 5x *1C-heterozygous

Results:
<table>
<tr><td colspan="4">Results compared to *1F/*1F:</td></tr>
<tr><td></td><td>no *1F</td><td>*1F-heterozygous</td><td>value for *1F/*1F</td></tr>
<tr><td rowspan="2">dose-corrected clozapine trough concentration</td><td>x 1.91 (S)</td><td>x 1.18 (NS)</td><td rowspan="2">0.67 ng/ml per mg/day</td></tr>
<tr><td colspan="2">Multivariate analysis showed the CYP1A2*1F polymorphism to be an independent predictor of dose-corrected clozapine trough concentration (S), explaining 24% of its variability.</td></tr>
<tr><td>clozapine trough concentration</td><td>x 1.91 (S)</td><td>x 1.31 (NS)</td><td>270.1 ng/ml</td></tr>
</table>
<table>
<tr><td colspan="3">Results for *1C-heterozygous compared to no *1C:</td></tr>
<tr><td></td><td>*1C-heterozygous</td><td>value for no *1C</td></tr>
<tr><td>dose-corrected clozapine trough concentration</td><td>x 0.87 (NS)</td><td>0.86 ng/ml per mg/day</td></tr>
<tr><td>clozapine trough concentration</td><td>x 0.76 (NS)</td><td>364.1 ng/ml</td></tr>
</table>
Note: In this study smoking was associated with dose-corrected clozapine trough concentration in univariate analysis, but not in multivariate analysis. So, smoking was not an independent predictor of dose-corrected clozapine trough concentration in this study.

Note: Genotyping was performed for *1C and *1F. These are the most</td>
<td>Authors' conclusions:
'Only the CYP-1A2*1 F polymorphism correlates significantly with the clozapine C0/D variation and could explain 24% of its variability. Our data support a critical role of the CYP-1A2 −163C>A on the variation of clozapine exposure in Tunisian schizophrenic patients. Considering its narrow therapeutic range, CYP1A2 genotyping combined with TDM of clozapine may improve efficacy and safety of this drug.'</td>
</tr>
</table>

<table>
<tr><td></td><td></td><td>important gene variants in this Tunisian population.</td><td></td></tr>
<tr><td>ref. 2
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

*1F/*1F : AA
*1A/*1F : AA
*1A/*1A : AA

*1C-heterozygous+homozygous: B</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:
- 10x *1A/*1L
- 6x *1F/*1L
- 6x *1F/*1V
- 5x *1L/*1L
- 4x *1A/*1A
- 4x *1A/*1V
- 3x *1L/*1V
- 2x *1F/*1F
- 2x *1A/*1F
- 2x *1V/*1V
- 2x *CF/*CF
- 1x *1A/*CF
- 1x *1F/*CF

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</td><td>homozygous</td><td>heterozygous</td><td>value for no gene variant</td></tr>
<tr><td rowspan="2">*1C-polymorphism</td><td>x 1.36</td><td>x 1.05</td><td rowspan="2">58.132 ng/ml per mg/kg</td></tr>
<tr><td colspan="2">NS for homozygous versus heterozygous versus no gene variant</td></tr>
<tr><td rowspan="2">*1D-polymorphism</td><td>x 1.08</td><td>x 0.88</td><td rowspan="2">65.477 ng/ml per mg/kg</td></tr>
<tr><td colspan="2">NS for homozygous versus heterozygous versus no gene variant</td></tr>
<tr><td rowspan="2">*1F-polymorphism</td><td>x 1.02</td><td>x 1.15</td><td rowspan="2">58.642 ng/ml per mg/kg</td></tr>
<tr><td colspan="2">NS for homozygous versus heterozygous versus no gene variant</td></tr>
<tr><td colspan="4">There was also no significant difference between the genotypes.</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 or between the genotypes (NS).</td></tr>
<tr><td colspan="4">Note: In this study, no differences in clozapine pharmacokinetics were found between smokers and non-smokers.</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 *1C-polymorphism compared to no *1C-polymorphism</td><td>OR = 3.86 (S)</td><td>NS</td><td>OR = 2.76 (NS before, but S after adjusting for false discove-</td><td>NS</td></tr>
</table></td><td>Authors' conclusion:
'Overall, CYP variants showed no effect on clozapine pharmacokinetics. The rs2069514 variant in homozygous genotype (also known as CYP1A2*1C/*1C) was associated with clozapine adverse reactions in Mexican patients with refractory psychosis (OR = 3.55) and demonstrated that this effect is doubled by concomitant alcohol consumption (OR = 7.9). Clinicians should be aware of this information before starting clozapine use, when treating patients with refractory psychosis, who are alcohol drinkers and carriers of this genetic variant in order to prevent clozapine-related adverse reactions.''</td></tr>
</table>

<table>
<tr><td>ref. 2, continuation</td><td></td><td>
<table>
<tr><td></td><td></td><td></td><td>ry rate)</td><td></td></tr>
<tr><td>homozygous *1C-polymorphism compared to heterozygous+ no *1C-polymorphism</td><td>OR = 5.71 (NS before, but S after adjusting for false discovery rate)</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>*1D-heterozygous+ homozygous: B</td><td>heterozygous+homozygous *1D-polymorphism compared to no *1D-polymorphism</td><td>NS</td><td>NS</td><td>OR = 4.94 (S)</td><td>NS</td></tr>
<tr><td>homozygous *1D-polymorphism compared to heterozygous+ no *1D-polymorphism</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>heterozygous+homozygous *1F-polymorphism compared to no *1F-polymorphism</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>*1A/*1F + NM: AA</td><td>homozygous *1F-polymorphism compared to heterozygous+ no *1F-polymorphism</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td colspan="5">Note: Because adjusting for false discovery rate should diminish the number of significant results and not increase it, it is surprising that two comparisons were NS before and S after adjusting for false discovery rate.</td></tr>
<tr><td colspan="5">Note: A binary logistic regression model with a calculated statistical power of 88.62% found a higher risk of adverse events in *1C-polymorphism carriers using alcohol during treatment than in *1C-polymorphism carriers not using alcohol.</td></tr>
</table>

NOTE: Genotyping was performed for the polymorphism present in *1C (-3860G>A) (also present in *1L), the polymorphism present in *1D (-2467delT) (also present in several other alleles, including *1L and *1V), the polymorphism present in *1F (-163C>A) (also present in several other alleles, including *1L and *1V), *4, and *8. *1D is not reported to result in decreased activity. The authors call the allele with both the *1C and *1F polymorphism *CF. *4 and *8 were not found in this patient group.
The determined gene variants are the most important gene variants in this Mexican population.</td><td></td></tr>
<tr><td>ref. 3
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:</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.
None of the patients received medication considered as strong CYP-1A2 inhibitor (i.e., fluvoxamine and ciprofloxacin). 47% of patients was smoker. CYP1A2 activity scores (1 for a *1A-allele and 1.5 for a *1F-allele) were corrected by multiplying them by 1.5 for current smokers. A NM phenotype was assigned to an activity score of 2, a UM phenotype to an activity score ≥ 2.5.
Clozapine plasma concentrations were adjusted for quantile–quantile (Q–Q) plots. P-values were corrected for multiple testing.

Genotyping:
- 12x NM (*1A/*1A)
- 54x UM (25x *1A/*1F, 29x *1F/*1F)
Genotype- and smoking-predicted phenotypes were: 6x NM, 60x UM.

Results:</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>31616047.

ref. 3, continuation</td>
<td>*1F/*1F : AA
*1A/*1F : AA
*1A/*1A : AA</td>
<td>
<table>
<tr><td colspan="3">Associations of genotype-predicted and genotype- and smoking-predicted CYP1A2 activity:</td></tr>
<tr><td rowspan="5">dose-corrected clozapine trough concentration</td><td rowspan="2">genotype-predicted</td><td>NS</td></tr>
<tr><td>Comparing patients above and below the clinical target clozapine blood concentration (i.e., 350 ng/mL) showed a trend for a higher genotype-predicted CYP1A2 activity among those with clozapine plasma levels less than 350 ng/L compared with those with levels above this threshold (p = 0.058) (NS).</td></tr>
<tr><td rowspan="3">genotype- and smoking predicted</td><td>S for a decrease with increasing CYP-1A2 activity</td></tr>
<tr><td>Comparing patients above and below the clinical target clozapine blood concentration (i.e., 350 ng/mL) showed a higher genotype-and smoking predicted CYP1A2 activity among those with clozapine plasma levels less than 350 ng/L compared with those with levels above this threshold (S).</td></tr>
<tr><td>Genotype- and smoking-predicted CYP1A2 activity explained 11% of the variation in clozapine trough concentration. However, smoking alone explained 17%.</td></tr>
<tr><td rowspan="3">symptom severity</td><td>genotype-predicted</td><td>NS</td></tr>
<tr><td rowspan="2">genotype- and smoking predicted</td><td>S for an increase with increasing CYP1A2 activity</td></tr>
<tr><td>Genotype- and smoking-predicted CYP1A2 activity explained 12% of the variation in symptom severity. However, smoking alone explained 13%.</td></tr>
</table>

NOTE: Genotyping was for *1F only. This is the most important gene variant in this Australian population.
</td>
<td></td>
</tr>
<tr>
<td>ref. 4
Pardiñas AF et al. Pharmacogenomic variants and drug interactions identified through the genetic analysis of clozapine metabolism. Am J Psychiatry 2019;176:477-86.
PMID: 30922102.</td>
<td>3

rs2472297C>T: A</td>
<td>
2989 patients were treated with clozapine. Plasma concentrations were determined 6-24 hours after the last dose. Plasma concentrations below the detection threshold of 0.05 mg/L (indicating nonadherence) were not included. Outliers outside the 99th percentile of plasma concentrations were also excluded. Removing data from a broader range of the extremes of the plasma concentration distributions did not meaningfully alter our results. The mean number of plasma concentration measurements per patient was 3.5.
A genome wide association was performed with the plasma concentrations adjusted for clozapine dose, time between dose and assay, and age at assay.
Data on smoking and co-medication were not available. Secondary sensitivity analyses controlling for proxy measures based on polygenic risk scores for cigarette smoking habits and weight were performed.

Results:
<table>
<tr><td colspan="2">Genome wide association results for plasma concentrations adjusted for clozapine dose, time between dose and plasma concentration measurement, and age at plasma concentration measurement:</td></tr>
<tr><td>(dose adjusted) clozapine plasma concentration</td><td>An association at rs2472297, an intergenic variant between CYP1A1 and CYP-1A2, was found (S).
Analysis of hepatocyte expression data did not relate this signal to any particular</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

<table>
<tr>
<td>ref. 4, continu-
ation</td>
<td></td>
<td>
<table>
<tr><td></td><td>gene, although rs2472297 has previously been associated with CYP1A2 activity on the basis of its effect on caffeine metabolite concentrations in a genome-wide association study. In the mixed-model analysis, this gene variant was shown to be associated with reduced clozapine plasma concentrations, with a proportion of variance explained of 1.47%.
Being heterozygous for the rs2472297 variant is associated with a reduction in clozapine plasma concentrations roughly equivalent to a decrease in clozapine by 50 mg/day, and homozygosity is equivalent to a reduction by 100 mg/day.</td></tr>
<tr><td>(dose adjusted) norclozapine plasma concentration</td><td>No association with a polymorphism in the CYP1A2 region was found.</td></tr>
<tr><td>clozapine/norclozapine metabolic ratio</td><td>No association with a polymorphism in the CYP1A2 region was found.</td></tr>
</table>

Note: PharmGKB considers rs2472297 to be a CYP1A1 instead of a CYP1A2 gene variant. It is located 10340 nucleotides more distant of the CYP1A2 coding sequence than the *1C polymorphism (rs2472297 = CYP1A2 -14200C>T).
</td>
<td></td>
</tr>
<tr>
<td>ref. 5
Na Takuathung M et al.
Impact of CYP1A2 genetic polymorphisms on pharmacokinetics of antipsychotic drugs: a systematic review and meta-analysis.
Acta Psychiatr Scand 2019;139:15-25.
PMID: 30112761.</td>
<td>3

*1F/*1F: AA
*1A/*1F: AA
*1A/*1A: AA

*1D: AA</td>
<td>Meta-analyses of 4 pharmacokinetic studies, including a total of 327 patients (44x no *1F, 133x *1F-heterozygous, 150x *1F/*1F). All included studies were of good quality, scoring 64-73 points of the maximum of 77 points on the 11-item quality scale for genetic studies Q-Genie. However, one of the included studies does not report dose-corrected plasma concentrations (Rajkumar 2013) and thus, should not have been included in the meta-analyses.
All 4 studies in the meta-analysis are also included in our risk analysis separately (Olsson 2015, Rajkumar 2013, Jaquenoud Sirot 2009, Kootstra-Ros 2005).
The review protocol was registered at the PROSPERO international prospective register of systematic reviews (CRD42017079514). Meta-analyses were performed with a random-effects model in case of significant heterogeneity between the studies and with a fixed-effect model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data exaction was standardised.
Publication bias analysis was performed for all comparisons.

Results:
<table>
<tr><td colspan="2">Standard mean difference of the (dose-corrected) clozapine plasma concentration compared to no *1F:</td></tr>
<tr><td>*1F-heterozygous</td><td>NS</td></tr>
<tr><td>*1F/*1F</td><td>NS</td></tr>
<tr><td colspan="2">For both comparisons, heterogeneity between the studies was lacking.</td></tr>
<tr><td colspan="2">For both comparisons, there were no indications for publication bias.</td></tr>
</table>

Note: The results of the meta-analyses investigating the pharmacokinetic effect of *1D were not included in this abstract, because *1D is a fully functional allele. In accordance with this, no effect of *1D on dose-corrected clozapine plasma concentration was found.
</td>
<td>Authors' conclusions:
'The pooled-effect estimates through meta-analyses of seven studies demonstrated no significant associations between the -163C>A or -2467delT polymorphism and clozapine or olanzapine concentrations in the blood.'</td>
</tr>
<tr>
<td>ref. 6
Vasudev K et al.
Genetic deter-</td>
<td>3</td>
<td>60 patients were on stable clozapine therapy for at least 6 months. The clozapine dose was 50-600 mg/day (mean 334 mg/day).
Metabolic syndrome was defined as having high waist circumference with 2 or more abnormal parameters including triglycerides, HDL cho-</td>
<td>Authors' conclusions:
'Pharmacogenetic markers in</td>
</tr>
</table>

minants of clozapine-induced metabolic side effects. Can J Psychiatry 2017;62:138-49. PMID: 27681143.

ref. 6, continuation

lesterol, blood pressure, and fasting glucose.
Co-medication affecting clozapine metabolism and/or body-mass index (BMI), and smoking were not excluded, but results were adjusted for the latter two. 35% of patients was smoker.
Allelic (variant allele versus wildtype allele), additive (homozygous variant versus heterozygous versus homozygous wildtype), dominant (homozygous variant + heterozygous versus homozygous wildtype), and recessive models (homozygous variant versus heterozygous + homozygous wildtype) were considered for each gene variant and the genetic model that best described the outcomes in linear regression models chosen. Multivariable linear regression models were used to evaluate the association of gene variants with drug concentration and metabolic side effects (BMI, blood sugar, and lipids), controlling for the confounding factors age, sex, concurrent medication with known effects on weight increase (quetiapine, valproate, lithium, risperidone, and olanzapine), smoking status, personal and family history of diabetes, and cardiovascular disease.
No correction for multiple testing was applied.

Genotyping (estimated based on the observed gene variant frequencies):

*1F:
- 30x *1F/*1F
- 25x *1F-heterozygous
- 5x no *1F

*1C:
- 56x no *1C
- 4x *1C-heterozygous

Results:

Effect of the genotypes on metabolic and kinetic outcomes (↓= decrease, ↑ = increase):

	*1C		*1F		
	heterozygous	no *1C	*1F/*1F	heterozygous	no *1F
BMI	NS	NS	NS	NS	NS
metabolic syndrome	NS	NS	↓ (S)	NS	NS
			There was an interaction between smoking and *1F/*1F (S), with smokers with *1F/*1F having a 4.6 times higher odds for metabolic syndrome, and non-smokers with *1F/*1F having a 0.08 times lower odds compared to no *1F.		
			There was no effect of smoking for all patients (all genotypes).		
fasting glucose	NS	NS	NS	NS	NS
lipid levels (including total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides)	NS	NS	NS	NS	NS
blood clozapine concentration	NS	NS	NS	trend for ↑ (p = 0.075) (NS)	NS
			There was an interaction between smoking and *1F-heterozygosity (S). Compared to no		

*1F/*1F: AA#

*1F/*1F, smokers: C

*1F/*1F, non-smokers: AA#

*1C-heterozygous: AA

*1A/*1F: AA
*1A/*1A: AA

*1A/*1F, smo-

genes encoding CYP2C19, leptin, leptin receptor, and HTR2C receptor were identified as main predictors of metabolic syndrome as well as BMI. …. In contrast to previous reports, CYP1A2 and ABCB1 genotype as well as smoking were not found to be significantly associated with clozapine level in our study. However, a significant interaction was observed between smoking and CYP1A2*1F g.-163 CA carrier status.'

<table>
<tr><td>ref. 6, continuation</td><td>kers: A

*1A/*1F, non-smokers: A

*1D: AA</td><td>
<table>
<tr><td></td><td></td><td></td><td>*1F, *1F-heterozygosity resulted in a 0.71 times lower clozapine level among smokers while enhancing exposure among non-smokers 2.8-fold.</td></tr>
<tr><td></td><td></td><td></td><td>There was no effect of smoking for all patients (all genotypes).</td></tr>
<tr><td colspan="4">Note: Metabolic syndrome was associated with clozapine blood levels (S), but BMI was not (NS).</td></tr>
</table>

Note: Genotyping was for *1C, *1D, and *1F. These are the most important gene variants in this Canadian population. Data for *1D were not included in the abstract, because *1D does not affect enzyme activity. In accordance with this, no effect of *1D on metabolic and kinetic outcomes was found.
</td><td></td></tr>
<tr><td>ref. 7
Huang HC et al.
Cigarette smoking has a differential effect on the plasma level of clozapine in Taiwanese schizophrenic patients associated with the CYP1A2 gene -163A/C single nucleotide polymorphism.
Psychiatr Genet 2016;26:172-7.
PubMed PMID: 27203225.</td><td>4

’*1F’/’*1F’: AA
*1A/’*1F’: AA
*1A/*1A: AA</td><td>143 patients were treated with clozapine 12.5-500 mg/day. Relevant co-medication was excluded. Patients were considered smokers if they regularly smoked at least 6 cigarettes per day. 65 patients smoked (45.4%). The percentage of smokers was higher among men (63%, 55 in 87 men) than among women (17%, 10 in 56 women). Correction took place for age, BMI, gender, smoking and clozapine dose.

Genotyping:
- 58x ’*1F’/’*1F’
- 73x *1A/’*1F’
- 12x *1A/*1A

Results:
<table>
<tr><td colspan="2">Dose-clozapine trough concentration of clozapine for ’*1F’/’*1F’ versus *1A/’*1F’ versus *1A/*1A:</td></tr>
<tr><td>All patients</td><td>No difference (NS)</td></tr>
<tr><td>Smokers</td><td>No difference (NS)</td></tr>
<tr><td>Non-smokers</td><td>Increase (NS for the trend and for ’*1F’/’*1F’ versus *1A/*1A)</td></tr>
<tr><td colspan="2">Smoking led to lower clozapine plasma concentrations in the total group (S). The percentage of smokers differed between the genotypes (34.4% for ’*1F’/’*1F’, 50.6% for *1A/’*1F’ and 66.6% for *1A/*1A).</td></tr>
<tr><td colspan="2">In the total group, there was a trend towards higher clozapine plasma concentrations in women (p = 0.063; NS). The percentage of women differed between the genotypes (39.7% for ’*1F’/’*1F’, 41.1% for *1A/’*1F’ and 25% for *1A/*1A).</td></tr>
</table>
<table>
<tr><td colspan="5">Effect of various parameters on clozapine plasma concentration and power of the effect analysis:</td></tr>
<tr><td colspan="2"></td><td>’*1F’/’*1F’</td><td>*1A/’*1F’</td><td>*1A/*1A</td></tr>
<tr><td rowspan="2">Smoking</td><td>effect</td><td>S</td><td>NS</td><td>NS</td></tr>
<tr><td>power</td><td>65%</td><td>16%</td><td>6%</td></tr>
<tr><td rowspan="2">Gender</td><td>effect</td><td>NS</td><td>S</td><td>NS</td></tr>
<tr><td>power</td><td>16%</td><td>51%</td><td>12%</td></tr>
<tr><td rowspan="2">Age</td><td>effect</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>power</td><td>18%</td><td>8%</td><td>16%</td></tr>
<tr><td rowspan="2">Dose</td><td>effect</td><td>S</td><td>S</td><td>S</td></tr>
<tr><td>power</td><td>100%</td><td>100%</td><td>61%</td></tr>
<tr><td rowspan="2">BMI</td><td>effect</td><td>trend, p = 0.056</td><td>trend, p = 0.092</td><td>NS</td></tr>
<tr><td>power</td><td>48%</td><td>39%</td><td>6%</td></tr>
</table>

Note: Genotyping was only performed for the *1F polymorphism (-163 C>A). In Asian people, this polymorphism mostly occurs in combination with other polymorphisms (*1L and *1V alleles).
</td><td>Authors’ conclusions:
‘Cigarette smoking has a significant impact on the plasma level of clozapine in Taiwanese schizophrenic patients carrying the homozygous −163A allele in the CYP1A2 gene. … It was also found that nonsmokers carrying the −163A allele tended to have higher plasma levels of clozapine. This tendency was not found in the individuals with smoking habits.’</td></tr>
</table>

<table>
<tr>
<td>ref. 8
de Brito RB et al.
The CYP1A2 -163C>A polymorphism is associated with super-refractory schizophrenia.
Schizophr Res 2015;169:502-3.
PubMed PMID: 26530626.</td>
<td>3

*1F/*1F : C
*1A/*1F : AA
*1A/*1A : AA#</td>
<td>Genotype frequencies were determined in 27 refractory and 27 super-refractory patients. Genotype frequencies were also compared to those of 64 healthy volunteers.
Refractory patients are treatment-resistant patients responding to clozapine. Super-refractory patients are treatment-resistant patients not responding to clozapine.
Co-medication affecting CYP1A2 and smoking were not excluded, but the percentage of smokers did not differ significantly between the genotype groups in patients. 43% of patients was smoker. Clozapine dose and the percentage of coffee drinkers also did not differ significantly between the genotype groups.

Genotyping:
refractory patients: - 9x *1F/*1F; - 11x *1A/*1F; - 7x *1A/*1A
super-refractory patients: - 20x *1F/*1F; - 7x *1A/*1F
healthy volunteers: - 26x *1F/*1F; - 22x *1A/*1F; - 16x *1A/*1A

Results:
The percentage of *1F/*1F was 2.2 and 1.8 fold higher in super-refractory patients than in refractory patients and healthy volunteers, respectively (S).
Note: Schizophrenic symptoms were measured with the Brief Psychiatric Rating Scale (BPRS). BPRS at baseline and BPRS at genetic testing were higher for *1F/*1F (S), but there was no difference in BPRS change (NS). The first two findings correspond with most *1F/*1F patients being super-refractory, the last finding does not.

Note: Genotyping was for *1F. This is the most important gene variant in this Brazilian population.</td>
<td>Authors' conclusions:
'Taken together, the results here suggest that the CYP1A2*1F polymorphism is associated with super-refractory schizophrenia, whereas smoking and coffee consumption do not seem associated with the super-refractoriness. Therefore, genotype testing for CYP1A2*1F may be a useful tool to predict the response to clozapine treatment.'</td>
</tr>
<tr>
<td>ref. 9
Olsson E et al.
Genetic and clinical factors affecting plasma clozapine concentration.
Prim Care Companion CNS Disord 2015;17;10.4088/PCC.14m01704.
PubMed PMID: 26137357.</td>
<td>3

*1F/*1F : AA#

(*1A/*1F + *1A/*1A): B</td>
<td>95 patients were treated with clozapine 50-875 mg/day (mean 324 mg/day). Relevant co-medication was not excluded. 54% of the patients were smokers. 32% also used other antipsychotics. Therapeutic drug monitoring was not routinely performed.

Genotyping:
*1F: - 9x *1A/*1A; - 35x *1A/*1F; - 51x *1F/*1F
*1D: - 86x *1A/*1A; - 7x *1A/*1D; - 2x *1D/*1D

Results:
*1F/*1F versus (*1A/*1F + *1A/*1A):
Dose-corrected trough concentration of clozapine: x 0.67 (S); The association remained significant after correction. The effect of *1F/*1F was similar to that of smoking. However, *1F/*1F, smoking and an MDR1 genotype together only predicted 16% of the variation in concentration. — Value for *1A/*1F + *1A/*1A: 2.1 ng.mg/mL
Increased fasting glucose concentration (>5.6 mmol/L): OR = 0.27 (S); Male gender and age increased the risk. There did not seem to be an association with plasma concentration. The authors reported that this may have been a coincidental</td>
<td>Authors' conclusions:
'There was a significant association between the rs762551 A allele of CYP1A2 and lower plasma clozapine concentration. Increased fasting glucose level was 3.7-fold more frequent in CC and CA genotypes than AA genotype (odds ratio = 0.27). There was no significant relation between higher fasting glucose levels and higher clozapine levels.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 9, continuation</td>
<td>1D: AA</td>
<td>
<table>
<tr><td></td><td>finding due to multiple testing.</td><td></td></tr>
<tr><td>Increased hip circumference</td><td>NS</td><td></td></tr>
</table>
<table>
<tr><td>*1D:</td></tr>
<tr><td>- There were no significant effects (NS)</td></tr>
</table>
Note: Alleles *1F, *1D and *1K were genotyped. *1K was not found in this patient group. *1D is a fully functional allele.
</td>
<td></td>
</tr>
<tr>
<td>ref. 10
Viikki M et al. CYP1A2 polymorphism -1545C > T (rs2470890) is associated with increased side effects to clozapine. BMC Psychiatry 2014;14:50. PubMed PMID: 24555493.</td>
<td>3

*1F/*1F : C
(*1A/*1A + *1A/*1F): AA#</td>
<td>
185 patients used clozapine for more than 3 months (mean 402 mg/day). The majority of patients (64%) used clozapine for more than 5 years. For 65% of the patients, clozapine was the only antipsychotic. The other 35% also used one or two other antipsychotics. Relevant co-medication was not excluded. 50% of the patients were smokers. Antipsychotic-induced side effects were measured using the Liverpool University Neuroleptic Side-Effect Rating Scale (LUNSERS).

Genotyping:
- 35x *1A/*1A
- 96x *1A/*1F
- 54x *1F/*1F

Results:
<table>
<tr><td colspan="3">*1F/*1F versus (*1A/*1A + *1A/*1F):</td></tr>
<tr><td></td><td></td><td>Value for CT+CC</td></tr>
<tr><td rowspan="3">Severity of the side effects, total</td><td>x 1.21 (S)</td><td rowspan="3">37.1 on the LUNSERS</td></tr>
<tr><td>There was also a significant effect for *1F/*1F versus *1A/*1F versus *1A/*1A (S), but the increase versus *1A/*1A (x 1.19) was not higher than that versus *1A/*1F (x 1.23).</td></tr>
<tr><td>Linear univariate analysis showed that the CYP1A2 genotype and the total antipsychotic dose together predicted 5.0% of the variation in severity of the side effects (S). Alternative models analysing clozapine monotherapy, antipsychotic combinations or regular smoking as factors were not significant (NS).</td></tr>
<tr><td rowspan="2">Use of mood stabilisers</td><td>x 1.9; OR = 2.63 (S)</td><td rowspan="2">23.3%</td></tr>
<tr><td>The most commonly used mood stabiliser was valproic acid.</td></tr>
<tr><td>Clozapine dose</td><td>trend (NS; x 1.1; p = 0.078)</td><td>390 mg</td></tr>
<tr><td colspan="3">No difference in:
- Severity of sympatheticotonia/tension (NS)
- Severity of depression/anxiety (NS)
- Severity of sedation (NS)
- Severity of orthostatic hypertension (NS)
- Severity of cutaneous side effects (NS)
- Severity of sexual side effects (NS)
- Severity of urinary side effects (NS)
- Clozapine trough concentration (NS)
- Norclozapine trough concentration (NS)
- Clozapine + norclozapine trough concentration (NS)
- Dose-corrected clozapine trough concentration (NS)
- Percentage of regular smokers (NS)
- Percentage of patients who used the CYP1A2 inducer carbamazepine (NS)</td></tr>
</table>
</td>
<td>Authors' conclusions:
'This study has identified an association between the CYP1A2 polymorphism −1545C > T (rs2470890) and the occurrence of more severe clozapine side effects. However, these results should be regarded as tentative and more studies of larger sample sizes will be required to confirm the result.'</td>
</tr>
</table>

<table>
<tr><td>ref. 10, continuation</td><td></td><td>- Percentage of patients using serotonin or serotonin/noradrenaline reuptake inhibitors (NS)
- Total antipsychotic dose (in chlorpromazine equivalents) (NS)

Note: Genotyping was performed for -1545C>T. -1545C>T is in complete linkage disequilibrium with -163C>A, the polymorphism in *1F.</td><td></td></tr>
<tr><td>ref. 11
Looman NMG et al.
Associatie van genetische variatie in CYP1A2 en UGT1A4 met metabole stoornissen bij gebruikers van clozapine en olanzapine [Association of genetic variation in CYP1A2 and UGT1A4 with metabolic disorders in users of clozapine and olanzapine].
PW Wetenschappelijk Platform 2013;7:a1310.</td><td>3

(*1A/*1F + *1F/*1F): A

*1A/*1A: AA#</td><td>70 patients used clozapine (25-800 mg/day; mean 337 mg/day). Relevant co-medication was not excluded. 61% of the patients were smokers.
ORs were corrected for age, gender, diagnosis, duration of disease, dose and smoking.
The use of CYP1A2 inducers and inhibitors in the patient group was too low to be able to correct for these. The number of patients was too low to consider smokers and non-smokers separately. Correction for the duration of clozapine usage was not possible as these data were missing for a large proportion of the patients.

Genotyping:
- 9x *1A/*1A
- 20x *1A/*1F
- 41x *1F/*1F

Results:
<table>
<tr><td colspan="5">Metabolic side effects versus *1A/*1A:</td></tr>
<tr><td></td><td>*1A/*1F</td><td>*1F/*1F</td><td>(*1A/*1F + *1F/*1F)</td><td>Value for *1A/*1A</td></tr>
<tr><td>Metabolic syndrome (OR_{corr})</td><td>NS</td><td>NS</td><td>NS</td><td>33%</td></tr>
<tr><td>HDL cholesterol</td><td></td><td></td><td>Decrease (S)</td><td></td></tr>
<tr><td>Uncontrolled glucose</td><td></td><td></td><td>NS</td><td></td></tr>
</table>

NOTE 1: The power calculation performed retrospectively showed that a significant difference would require a much larger number of patients (~300).

NOTE 2: Genotyping was performed for *1F and *1C. *1C was not found in the patient population.</td><td>Authors' conclusions:
'This study showed that there is no relationship between genetic variation in CYP1A2 and UGT1A4 and the occurrence of metabolic syndrome in users of clozapine and olanzapine.'</td></tr>
<tr><td>ref. 12
Kohlrausch FB et al.
The CYP1A2 -163C>A polymorphism is associated with clozapine-induced generalized tonic-clonic seizures in Brazilian schizophrenia patients.
Psychiatry Res 2013;209:242-5.
PubMed PMID: 23601795.</td><td>3

*1F: C
*1A:</td><td>106 patients were treated with a stable clozapine dose (100-900 mg/day; mean 543.5 mg/day). 24 patients developed clozapine-induced generalised epileptic seizures. None of the patients had a history of generalised epileptic seizures.
Relevant co-medication was not excluded. In logistic regression analysis, corrections were made for the dose (≥ 650 mg/day), presence of co-medication and smoking (yes or no). These three parameters had no significant effect on the risk of seizures. The results were not corrected for the duration of the treatment, although this was significantly longer for patients who developed seizures.

Genotyping:
- 15x *1A/*1A
- 4x *1A/*1C
- 35x *1A/*1F
- 1x *1C/*1C
- 13x *1C/*1F
- 38x *1F/*1F

*1F-allele:
- The incidence of *1F was higher in patients who developed seizures compared to patients who did not develop seizures (S) (73% versus</td><td>Authors' conclusion:
"We found the CYP1A2 *1F/*1F genotype to be significantly associated with seizures and no relationship was observed with combinations of *1F and *1C alleles."</td></tr>
</table>

<table>
<tr><td>ref. 12, continuation</td><td>AA#

*1F/*1F: C

*1A/*1C: AA
*1C/*1C: AA</td><td>54%; OR = 2.27; 95% CI: 1.073-5.013)
- There were no significant differences in distribution of the genotype groups (no *1F, heterozygous *1F and *1F/*1F) over the patients with and without seizures (NS)
- Compared to (no *1F + heterozygous *1F), *1F/*1F was more common in patients with seizures than in patients without seizures (S) (OR_{corr} = 2.89; 95% CI: 1.04-8.02)

*1C-allele:
- There were no differences in the frequency of *1C and the corresponding genotype groups between patients who developed seizures and those who did not (NS)</td><td></td></tr>
<tr><td>ref. 13
Rajkumar AP et al.
Association between CYP1A2 gene single nucleotide polymorphisms and clinical responses to clozapine in patients with treatment-resistant schizophrenia.
Acta Neuropsychiatr 2013;25:2-11.
PMID: 26953068.</td><td>3

*1F/*1F: AA
*1A/*1F: AA
*1A/*1A: AA

*1C-heterozygous: AA</td><td>101 patients were treated with clozapine for a period of 4-174 months (median 28 months). The clozapine dose was 100-650 mg/day (median 350 mg/day). Patients were on a stable dose for at least 12 weeks. Non-response to clozapine was defined as a total score > 35 on the Brief Psychiatric Rating Scale (BPRS). Cognitive status was evaluated with Addenbrooke's Cognitive Examination (ACE-R), a brief cognitive test. Disability was quantified with the World Health Organisation Disability Assessment Scale II.
Adverse effects related to clozapine observed in the study were hypersomnolence (76%), sialorrhea (47%), nausea or vomiting (21%), constipation (21%), erectile dysfunction (28%), dyslipidaemia (12%), clozapine-related seizures (9%), nocturnal enuresis (6%) and obesity (15%).
Trough plasma concentrations (12 hours after dosing) were determined.
None of the patients used carbamazepine or oral contraceptive pills, but other comedication affecting CYP1A2 was not excluded and not adjusted for. 17% of patients smoked more than 1 pack/day, and this was associated with non-response (as were past history of catatonia, higher clozapine doses, higher disease scores (BPRS) and lower cognitive scores (ACE-R)).
Based on the *1F frequency of 0.386 in the Asian population, it was calculated that 34 cases of clozapine non-responders would be needed for 80% power to find an effect with an OR of 2.5. With 44.3% of treatment-resistant patients being clozapine non-responders, this would amount to 77 patients.

Genotyping:
*1F:
- 37x *1F/*1F
- 41x *1F-heterozygous
- 23x no *1F
*1C:
- 81x no *1C
- 18x *1C-heterozygous
- 2x *1C/*1C

Results:
<table>
<tr><td colspan="4">Results for homozygous variant versus heterozygous versus no variant:</td></tr>
<tr><td></td><td colspan="2">gene variant</td><td rowspan="2">value for no *1F/no *1C</td></tr>
<tr><td></td><td>*1F</td><td>*1C</td></tr>
<tr><td rowspan="3">% of non-responders</td><td>NS</td><td>NS</td><td rowspan="3">48%/ 36%</td></tr>
<tr><td colspan="2">Multiple logistic regression analyses, adjusting for the effects of various clinical variables, including the serum clozapine levels, confirmed the absence of an effect.</td></tr>
<tr><td colspan="2">The absence of an effect was confirmed for the following 3 definitions of non-response:
- total BPRS score ≥ 38 (worst</td></tr>
</table></td><td>Authors' conclusions:
'As CYP1A2 gene SNP do not help to predict the clinical response to clozapine, routine screening for them prior to start clozapine is currently unwarranted.'</td></tr>
</table>

ref. 13, continuation				quartile) - at least one of the five selected BPRS items for suspiciousness, hallucinatory behaviours, grandiosity, conceptual disorganisation and unusual thought content scoring ≥ moderate - at least two of these five selected BPRS items scoring ≥ moderate		
				Patients who smoked more than 20 cigarettes a day (n = 17) did not differ in their clinical responses to clozapine depending on their *1F genotypes (NS). Multivariate analyses adjusting for the effects of age, oral dose, and body mass index confirmed the absence of an effect.		
			adverse events	NS	NS	
				Multiple logistic regression analyses, adjusting for the effects of other clinical variables, including the serum clozapine levels, confirmed the absence of an effect.		
			schizophrenia symptoms (BPRS score)	NS	NS	36.6/ 34.5
				Appropriate multiple quantile regression analyses, adjusting for the effects of other clinical variables, including the serum clozapine levels, confirmed the absence of an effect.		
			World Health Organisation Disability Assessment-II Scale score	NS	NS	17.4/ 17.8
				Appropriate multiple quantile regression analyses, adjusting for the effects of other clinical variables, including the serum clozapine levels, confirmed the absence of an effect.		
			Addenbrooke's cognitive examination-revised score	NS	NS	61.1/ 62.4
				Appropriate multiple quantile regression analyses, adjusting for the effects of other clinical variables, including the serum clozapine levels, confirmed the absence of an effect.		
			serum clozapine concentration	NS	NS	602/ 544 ng/ml
				Appropriate multiple quantile regression analyses, adjusting for the effects of other clinical variables confirmed the absence of an effect.		
				Patients who smoked more than 20 cigarettes a day (n = 17) did not differ in their serum clozapine levels depending on their *1F genotypes (NS). Multivariate analyses adjusting for the effects of age, oral dose, and body mass index confirmed the absence of an effect.		
		Note: Genotyping was for *1C, *1D, *1E, and *1F. These are the most				

ref. 13, continuation	*1D: AA *1E: AA	important gene variants in this South Indian population. Data for *1D and *1E were not included in the abstract, because these gene variants do not affect enzyme activity. Accordingly, no significant kinetic or clinical effects were found for these gene variants.	
ref. 14 Ferrari M et al. Association between CYP-1A2 polymorphisms and clozapine-induced adverse reactions in patients with schizophrenia. Psychiatry Res 2012;200:1014-7. PubMed PMID: 22901441.	3 *1C: AA *1F: AA *1A: AA (*1A/*1A + *1A/*1C + *1C/*1C + *1C/*1F): C (*1F/*1F + *1A/*1F): AA[#]	12 patients with grade 3-4 clozapine-induced side effects that necessitated withdrawal (n=5) or permanent dose-reduction of clozapine (n=7) were compared to 22 patients without side effects who were treated with clozapine for at least 1 year. There were no significant differences between the two groups in the clozapine dose (mean 374 mg/day) and the percentage of patients who used co-medication or smoked. Co-medication was excluded, with the exception of benzodiazepines. Side effects were primarily neurological (58%) and cardiovascular (16%) in nature. The most common side effect was sedation (21%). Results were not corrected for smoking, dose or use of benzodiazepines. Genotyping: *1C: - 21x *1A/*1A - 12x *1A/*1C - 1x *1C/*1C *1F: - 8x *1A/*1A - 19x *1A/*1F - 7x *1F/*1F Combined: - 14x low activity (*1A/*1A, *1A/*1C, *1C/*1C, *1C/*1F) - 20x high activity (*1F/*1F, *1A/*1F) Results: - No significant association between *1C and *1F and side effects (NS) - No significant association between *1C and *1F and CYP1A2 mRNA levels in lymphocytes (NS) - Low-activity genotypes were more common in patients with than in patients without side effects (67% versus 27%) (S) - CYP1A2 mRNA levels in lymphocytes were lower in patients with low-activity genotypes than in those with high-activity genotypes (0.036 versus 0.311 normalised to 18S ribosomal RNA) (S) CYP1A2 mRNA levels in lymphocytes were lower in patients with side effects than in those without (0.047 versus 0.285 normalised to 18S ribosomal RNA) (S). A threshold value of 0.076 predicted clozapine-induced side effects with a sensitivity of 83%, a specificity of 63% and a positive probability ratio of 7.64.	Authors' conclusion: "Patients with clozapine-induced adverse drug reactions had a higher frequency of CYP1A2 low activity allele combinations (8/12; 67%, P=0.019)."
ref. 15 Lee ST et al. Association study of 27 annotated genes for clozapine pharmacogenetics: validation of pre-existing studies and identification of a new candidate gene, ABCB1, for treatment response. J Clin Psychopharmacol 2012;32:441-8.	4 *1W: AA rs2069522: AA '*1F': AA *1A: AA	96 patients were treated with clozapine for more than 6 months. Co-medication with CYP1A2 inhibitors and other anti-psychotic drugs was excluded. Correction took place for smoking. Genotyping was performed for 8 CYP1A2 polymorphisms, including -163C>A, which occurs - amongst others - in *1F. Results: - 2 out of the 8 polymorphisms (*1W and rs2069522) exhibited a significant association with dose and weight-corrected plasma concentrations of clozapine and norclozapine (S). However, this association was no longer significant after correction for multiple statistical testing (NS). Note: Genotyping was performed for polymorphisms in *1B, *1E, *1F, *1K, *1W, *4, *5 and rs206952. Polymorphisms were selected if their allele frequency was greater than 0.05 in Chinese, Japanese or Korean individuals. As the polymorphism in *1F in Asian people mainly occurs in *1K and *1W, a possible explanation for a missing association with this polymorphism is that *1K and *1W mask the effect of	Authors' conclusion: "Among the pharmacokinetic-related single nucleotide polymorphisms, rs2069521 and rs2069522 in CYP1A2 for clozapine/(dose/weight) and norclozapine/(dose/weight) showed borderline associations that were insignificant after correction for multiple testing."

PubMed PMID: 22722500.		*1F.	
ref. 16 Bolla E et al. Are CYP1A2 *1F and *1C associated with clozapine tolerability?: a preliminary investigation. Psychiatry Res 2011;189:483. PubMed PMID: 21481946.	2 *1A/*1C: C *1C/*1C: C *1F/*1F: AA	3 patients with grade 3 clozapine-induced side effects that resulted in withdrawal of treatment were compared to 5 patients without side effects who were treated with clozapine for at least 1 year. The control patients were comparable with regard to clinical characteristics, age, gender and smoking. The patients with side effects were smokers, received no co-medication with drugs that can affect CYP1A2 activity, but were initially receiving diazepam. The clozapine doses (150 mg/day and twice 300 mg/day) were lower than among the control patients (450 mg/day). The side effects consisted primarily of neurological effects, such as severe sedation. These effects could not be explained by co-morbidity or co-medication and disappeared soon after dose reduction or withdrawal of clozapine. Of the patients with side effects, one was *1C/*1C and the other two were *1A/*1C. All control patients were *1F/*1F. CYP1A2 mRNA levels in lymphocytes among patients with side effects were less than 1/30th of the levels in the control patients (S).	Authors' conclusion: "Our results suggest that concomitant absence of CYP1A2 *F and presence of CYP1A2*C could generate CYP1A2 phenotypes which could induce lower mRNA expression and predispose to clozapine intolerance."
ref. 17 Jaquenoud Sirot E et al. ABCB1 and cytochrome P450 polymorphisms: clinical pharmacogenetics of clozapine. J Clin Psychopharmacol 2009;29:319-26. PubMed PMID: 19593168.	3 *1F: AA *1A: AA	75 patients were treated with a stable dose of clozapine (25-800 mg/day; median 250 mg/day). 17 patients also received the strong CYP1A2 inhibitor fluvoxamine (25-300 mg/day). No significant effect on the plasma concentrations of norclozapine/clozapine was found for other possibly relevant co-medications (6x sertraline, 3x paroxetine, 1x fluoxetine, 3x levomepromazine, 2x amlodipine, 1x phenytoin, 1x omeprazole). 45 patients were smokers. There was no simultaneous correction for smoking and fluvoxamine. There were no serious side effects, but excessive salivation and weight gain were common. Genotyping: - 8x *1A/*1A - 31x *1A/*1F - 34x *1F/*1F Results: - Differences in dose-corrected plasma concentrations of clozapine, norclozapine and clozapine + norclozapine were not found between the different genotypes in the total group, in the 58 patients who did not receive fluvoxamine and in the 45 smokers (NS) There were strong correlations between CYP1A2 activity and dose-corrected plasma concentrations of clozapine, norclozapine and clozapine + norclozapine in the total group, the group with fluvoxamine and the group without fluvoxamine (S). Note: Genotyping was only performed for *1F.	Authors' conclusion: "CYP1A2 activity and dose-corrected trough steady-state plasma concentrations of clozapine correlated significantly, with no influence of the CYP1A2*1F genotype."
ref. 18 Melkersson KI et al. Impact of CYP1A2 and CYP2D6 polymorphisms on drug metabolism and on insulin and lipid elevations and insulin resistance in clozapine-treated patients. J Clin Psychiatry 2007;68:697-704.	3 (*1C,	17 patients were treated with clozapine (100-600 mg/day; median 400 mg/day) for at least 0.7 years. Co-medication with an effect on glucose or lipid metabolism and known inhibitors or inducers of CYP1A2 were excluded. 6 patients were smokers. There was no significant effect of smoking on the plasma concentrations of clozapine and norclozapine. Genotyping: Of the 34 alleles, 15 had expected reduced activity (7x no *1F, 5x *1D, 1x *1C, 1x *1E and 1x *1K). There is no knowledge on the effect of the polymorphism on the activity of *1D and *1E. Genotypes: - 4x two alleles with expected reduced activity (*1C, *1D, *1E, *1K, no *1F) - 7x one allele with expected reduced activity (*1C, *1D, *1E, *1K, no *1F) - 6x two alleles with expected normal/increased activity (*1F, no *1C, no *1D, no *1E, no *1K)	Authors' conclusion: "CYP1A2 variants *1C and *1D seem to be associated with higher serum clozapine concentrations and an increased risk of developing insulin and lipid elevations and insulin-resistance on a given dose of clozapine."

PubMed PMID: 17503978. **ref. 18, continuation**	*1D, *1E, *1K, no *1F): A	(2 alleles with expected reduced activity) compared to (2 alleles with expected normal/increased activity): - ~2-Fold increase in the median dose-corrected plasma concentrations of clozapine and norclozapine (S) - No differences in plasma concentrations of insulin, C-peptide, triglyceride and cholesterol and in insulin resistance (NS) (1 allele with expected reduced activity) compared to (2 alleles with expected normal/increased activity): - Non-significant increase in the median dose-corrected plasma concentrations of clozapine and norclozapine (NS) - No differences in plasma concentrations of insulin, C-peptide, triglyceride and cholesterol and in insulin resistance (NS) Patients with an increased insulin concentration: - Are more likely to be carriers of *1C and *1D (all 5 carriers had increased insulin concentrations) (S) - Are no more likely to be carriers of (no *1F), *1E and *1K than patients with normal insulin concentrations (NS)	
ref. 19 Kootstra-Ros JE et al. The cytochrome P450 CYP-1A2 genetic polymorphisms *1F and *1D do not affect clozapine clearance in a group of schizophrenic patients. Ann Clin Biochem 2005;42:216-9. PubMed PMID: 15949157.	4 *1F: AA *1A: AA *1D: AA or A	*1C and *1D were determined for the 58 patients from Van der Weide, 2003 without co-medication that affects the CYP1A2 activity. 33 patients were smokers. Genotyping: Smokers: / Non-smokers: *1F: / *1F: - 3x *1A/*1A / - 1x *1A/*1A - 12x *1A/*1F / - 14x *1A/*1F - 18x *1F/*1F / - 10x *1F/*1F *1C: / *1C: - 32x *1A/*1A / - 25x *1A/*1A - 1x *1A/*1C *1D: / *1D: - 28x *1A/*1A / - 24x *1A/*1A - 2x *1A/*1D / - 1x *1A/*1D - 1x *1D/*1D Results: *1F: - Dose-corrected plasma concentrations of clozapine increase non-significantly in smokers and decrease non-significantly in non-smokers as a function of the number of *1F alleles (NS). The same applies to dose and weight-corrected plasma concentrations in smokers. - Multivariate analysis only revealed an effect of *1F and/or *1D on dose-corrected plasma concentrations in smokers (S). However, this was non-significant after exclusion of the patient with the *1D/*1D genotype (NS). This patient also had a *1C polymorphism. *1D: - Dose-corrected plasma concentrations of clozapine decrease non-significantly in smokers as a function of the number of *1F alleles (NS). - Multivariate analysis only revealed an effect of *1F and/or *1D on dose-corrected plasma concentrations in smokers (S). However, this was non-significant after exclusion of the patient with the *1D/*1D genotype (NS). This patient also had a *1C polymorphism. *1C: - The patient who was heterozygous for *1C was also homozygous for *1D. This is possibly a case of *1D/*1L. The *1L allele contains the polymorphisms for *1C, *1D and *1F. - The patient had a high dose-corrected plasma concentration of	Authors' conclusion: "Multivariate analysis of variance did not reveal any significant correlations between CYP1A2 genotypes and clozapine clearance in these subjects, although a possible effect of the *1D allele cannot be excluded in this study."

ref. 19, continuation	*1C: -	clozapine (1.90 ng/mL per mg compared to the mean of 0.83 ng/mL per mg for the other patients) and a high dose and weight-corrected plasma concentration (153 ng.kg/mL per mg), but a cause other than a genetic cause could not be ruled out.	
ref. 20 van der Weide J et al. The effect of smoking and cytochrome P450 CYP1A2 genetic polymorphism on clozapine clearance and dose requirement. Pharmacogenetics 2003;13:169-72. PubMed PMID: 12618594.	3 *1F/*1F: AA (*1A/*1F + *1A/*1A): AA	80 patients, including 45 smokers, were treated with a stable dose of clozapine (25-700 mg/day) for at least 3 months. 21 patients received co-medication that could possibly affect clozapine metabolism, such as fluvoxamine or carbamazepine. Genotyping: - 36x *1F/*1F, including 22 smokers - 44x (no *1F/*1F), including 23 smokers Results: *1F/*1F versus (no *1F/*1F:): - No differences in dose-corrected plasma concentrations of clozapine in the group of smokers or in the group of non-smokers (NS) - No differences in the daily dose of clozapine in the group of smokers (NS). The same applies to the daily dose of clozapine in the group of smokers without co-medication, non-smokers without co-medication, smokers with therapeutic plasma concentrations of clozapine and non-smokers with therapeutic plasma concentrations of clozapine (NS). NOTE: genotyping was only performed for *1F.	Authors' conclusion: "Neither among smokers, nor among nonsmokers mean concentration/dose ratios and daily doses did vary significantly between patients with the different CYP1A2 genotypes. The results show that clozapine clearance and daily dose requirement are strongly associated with smoking behaviour, while the CYP1A2 genetic polymorphism seems to have no significant clinical effect."
ref. 21 Basile VS et al. Genetic dissection of atypical antipsychotic-induced weight gain: novel preliminary data on the pharmacogenetic puzzle. J Clin Psychiatry 2001;62:45-66. PubMed PMID: 11603885.	3 *1F: AA *1A: AA	Treatment with clozapine was started in 70 patients (dose unknown, adjustment to plasma concentration of 200-420 ng/mL). Relevant co-medication and smoking were not excluded. Correction was performed for gender, ethnicity, body weight before the start of the treatment and whether or not patients responded to clozapine. Genotyping: - 35x *1F/*1F - 23x *1A/*1F - 12x *1A/*1A *1F/*1F versus *1A/*1F versus *1A/*1A: - No difference in increase in body weight during treatment for 6 weeks (NS) The authors stated that definitive conclusions could not be drawn from the study results due to the limited study size. NOTE: genotyping was only performed for *1F.	Authors' conclusion: "In general, there were no other observable genetic associations (with clozapine induced weight gain) for the remaining candidate genes tested (5-HT1A, 5-HT2A, histamine Ha and H2 receptor genes, and CYP1A2)."

AA[#]: the allele has a significant effect, but this effect is favourable instead of unfavourable.

Risk group	-

Comments:

- Cases and case series in which the patients were not compared to similar patients with the wild-type genotype were not included in the risk analysis, because they do not contribute sufficiently to the evidence. CYP1A2 activity is strongly affected by non-genetic factors. In addition, because of the prevalence of *1F/*1F and *1F-heterozygotes being much higher than 5%, finding one of these genotypes in a case does not suggest significance (i.e. probability < 0.05).
  This involves the following cases:
  - 1 case with low plasma concentrations of clozapine and lack of effectiveness being a *1F-carrier (n = 1, smoker)

- 1 case with high plasma concentrations of clozapine and adverse events with *1F/*1L (n = 1, comedication with venlafaxine, which is known to increase the clozapine plasma concentration, at the time of plasma concentration measurements)
- 1 case series and 1 case with low plasma concentrations of clozapine and lack of effectiveness with *1F/*1F (n=4, all smokers)
- 1 case with a strong increase in plasma concentrations of clozapine and an increase in the incidence of side effects with smoking cessation with *1F/*1F (n=2)
- 1 case with a high plasma concentration of clozapine and a side effect of asymptomatic pancreatitis with (*1F/*1F) (n=1, heavy smoker)
- 1 case with a high plasma concentration of clozapine and side effects with *1A/*7 (n=1, non-smoker)

The study of Islam 2021 (Islam F et al. Contributions of cholinergic receptor muscarinic 1 and CYP1A2 gene variants on the effects of plasma ratio of clozapine/N-desmethylclozapine on working memory in schizophrenia. J Psychopharmacol 2021;35:31-9. PMID: 33143542) was not included, because plasma concentrations were not corrected for dose.

The study of Ruan 2019 (Ruan CJ et al. Clozapine metabolism in East Asians and Caucasians: a pilot exploration of the prevalence of poor metabolizers and a systematic review. J Clin Psychopharmacol 2019;39:135-44. PMID: 30811372) was not included, because only the effect of gene variants on the plasma concentration of clozapine+norclozapine was investigated.

Date of literature search: 4 August 2021.

	Genotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	*1A/*1F	4 B	no	no	13 September 2021
	NM	4 B	no	no	
	*1C-heterozygote	4 C	no	no	
	*1C/*1C	3 C	no	no	
	IM	-	no	no	
	PM	-	no	no	

Mechanism:

Clozapine is mainly converted by CYP1A2 to the active metabolite N-desmethylclozapine (norclozapine). The activity of norclozapine is low and not considered clinically relevant. Clozapine is also metabolised to a limited extent by CYP-3A4 and possibly by CYP2C19 and CYP2C9. Norclozapine also appears to be metabolised primarily by CYP1A2: low CYP1A2 activity results in high plasma concentrations of norclozapine.

The NVZA (Dutch association of hospital pharmacists) recommends to titrate to a clozapine trough plasma concentration >350 µg/L with an upper limit of 700 µg/L in case of insufficient effectiveness. A better effectiveness has been observed at a concentration >350 µg/L. Concentrations >1000 µg/L are toxic. In literature, a therapeutic range of clozapine of 350-600 ng/mL with toxic concentrations > 1,000 ng/mL is mentioned (Hiemke C et al. Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017. Pharmacopsychiatry 2018; 51:9-62).