CYP2C19: amitriptyline 7024 to 7026

AUC = area under the plasma concentration-time curve, Cl_{or} = oral clearance, C_{ss} = steady-state concentration, IM = intermediate metaboliser (*1/*2, *1/*3, *2/*17, *3/*17) (reduced CYP2C19 enzyme activity), MR = metabolic ratio, NM = normal metaboliser (*1/*1, *1/*17) (normal CYP2C19 enzyme activity), NS = non-significant, PM = poor metaboliser (*2/*2, *2/*3, *3/*3) (absent CYP2C19 enzyme activity), S = significant, SmPC = summary of product characteristics, TCA = tricyclic antidepressant, UM = ultra-rapid metaboliser (*17/*17) (increased CYP2C19 enzyme activity).

Disclaimer: The Pharmacogenetics Working Group of the KNMP formulates the optimal recommendations for each phenotype group based on the available evidence. If this optimal recommendation cannot be followed due to practical restrictions, e.g. therapeutic drug monitoring or a lower dose is not available, the health care professional should consider the next best option.

Brief summary and justification of choices:

Amitriptyline is mainly converted by CYP2C19-mediated N-demethylation to the active metabolite nortriptyline. Both amitriptyline and nortriptyline are metabolised by CYP2D6 to 10-hydroxy metabolites, predominantly E-10-hydroxy metabolites. Amitriptyline is approximately three times as potent as E-10-OH-amitriptyline. Nortriptyline is approximately twice as potent as E-10-OH-nortriptyline.

N-oxidation and N-glucuronidation of amitriptyline also take place. Nortriptyline is converted by CYP2D6 and CYP2C19 to the inactive metabolite didesmethylamitriptyline (desmethylnortriptyline).

The therapeutic range of amitriptyline is a sum concentration of amitriptyline and nortriptyline of 100-300 ng/ml and values higher than 400 ng/ml are considered to be toxic. An upper limit is indicated for the therapeutic range of nortriptyline (50-150 ng/ml), but not for the therapeutic range of amitriptyline (> 50 ng/ml).

Genetic variants in CYP2C19 can result in a decreased CYP2C19 enzyme activity (intermediate metabolisers (IM)), an absent CYP2C19 enzyme activity (poor metabolisers (PM)) or an increased CYP2C19 enzyme activity (ultra-rapid metabolisers (UM)).

IM and PM: All 7 studies including kinetics showed that a genetically decreased CYP2C19 enzyme activity (IM and PM) increased the amitriptyline/nortriptyline ratio by increasing the amitriptyline exposure and decreasing the nortriptyline exposure (Ryu 2017, De Vos 2011, van der Weide 2005, Steimer 2005, Steimer 2004, Jiang 2002, Shimoda 2002). However, IM and PM hardly influenced the sum concentration of amitriptyline+nortriptyline, that determines efficacy and side effects. Compared to NM, the sum exposure increased with a weighted mean of 7% for IM and a weighted mean of 15% for PM. Accordingly, the two studies investigating response (Atasayar 2016 and Steimer 2005) and the two studies investigating adverse drug reactions (Ryu 2017 and Steimer 2005), did not find a significant effect of IM and/or PM compared to NM. The largest study on adverse drug reactions and the only one including patients and repeated dosing analysed only 1 PM (Steimer 2005). However, for 18 IM + 1 PM, this study showed a trend for a decrease in adverse drug reactions compared to NM, making it unlikely that a significant increase in adverse drug reactions due to a amitriptyline/nortriptyline imbalance would have been found for PM if more PM would have been studied. The KNMP Pharmacogenetics Working Group concludes that there is a gene-drug interaction for IM and PM, but adjustment of therapy is not required (yes/no-interactions).

UM: The only study investigating 8 UM (De Vos 2011) showed contradictory results. The percentage of patients with supratherapeutic plasma concentrations (nortriptyline > 150 ng/ml) was significantly higher for UM compared to *1/*1 if only CYP2D6 NM were analysed, but the nortriptyline plasma concentration did not differ significantly between UM and *1/*1 (respectively 83 and 71 ng/ml). The dose-corrected amitriptyline+nortriptyline concentration could be calculated for 5 UM. This sum concentration was 78% of that for NM. A dose increase of 29% would be required to correct for this decrease. However, the upper limit of the nortriptyline therapeutic range (150 ng/ml) is considerably lower than that of the amitriptyline+nortriptyline therapeutic range (300 ng/ml). This means that at higher nortriptyline/amitriptyline ratios as occur in UM, the upper limit of the nortriptyline therapeutic range might be reached at lower doses than the upper limit of the amitriptyline+nortriptyline therapeutic range, making it uncertain whether recommendation of a dose increase would improve or worsen the therapy. The KNMP Pharmacogenetics Working Group concludes that there is a gene-drug interaction, but that there is insufficient evidence to recommend adjustment of therapy for UM (yes/no-interaction).

You can find a detailed overview of the observed kinetic and clinical effects per phenotype in the background information text of the gene-drug interactions on the KNMP Kennisbank. You might 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
Ryu S et al.
A study on CYP-2C19 and CYP2D6 polymorphic effects on pharmacokinetics and pharmacodynamics of amitriptyline in healthy Koreans.
Clin Transl Sci 2017;10:93-101.
PubMed PMID: 28296334.</td><td>3

PM: A

IM: AA</td><td>24 healthy volunteers, selected for their CYP2C19 genotype, received a single dose of amitriptyline 25 mg. Volunteers and investigators were blinded to the CYP2C19 genotype.
The subjects rated dry mouth and drowsiness on visual analogue scales before and 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 96 hours after dosing. Medication did not cause significant drowsiness or a change in blood pressure.
Eight adverse events occurred in the 24 volunteers, of which four were considered amitriptyline-related (1x dry eyes, 2x headache, 1x head heaviness). All events were mild and fully recovered.
Co-medication and smoking were excluded.

Genotyping:
- 8x NM
- 10x IM
- 6x PM

Results:
<table>
<tr><td colspan="4">Results compared to NM+IM or NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM+IM or NM</td></tr>
<tr><td>dry mouth</td><td colspan="2">no difference between groups (NS)</td><td></td></tr>
<tr><td>drowsiness</td><td colspan="2">no difference between groups (NS)</td><td>no significant increase</td></tr>
<tr><td>increase in pulse rate</td><td colspan="2">no difference between groups (NS)</td><td></td></tr>
<tr><td>change in blood pressure</td><td colspan="2">no difference between groups (NS)</td><td>no significant change</td></tr>
<tr><td>AUC amitriptyline (in ng.h/ml)</td><td>x 1.78 (S)</td><td></td><td>NM+IM: 268.26</td></tr>
<tr><td>AUC nortriptyline (in ng.h/ml)</td><td>x 0.54 (S)</td><td></td><td>NM+IM: 234.03</td></tr>
<tr><td>AUC amitriptyline + AUC nortriptyline (in ng.h/ml)</td><td>x 1.20 (NS (significance not determined))</td><td></td><td>NM+IM: 502.28</td></tr>
<tr><td>AUC ratio amitriptyline/nortriptyline</td><td>x 4.03 (S)</td><td>x 1.58 (NS)</td><td>NM: 0.85</td></tr>
</table>

NB: Genotyping was for *2, *3 and *17. These are the most important gene variants in this Korean population. None of the patients had *17.</td><td>Author’s conclusion:
”The extent of N-demethylation of amitriptyline significantly decreased in subjects carrying two nonfunctional alleles of CYP2C19. ... The gene variations of CYP2C19 and CYP2D6 did not change the pharmacodynamic effect.”</td></tr>
<tr><td>ref. 2
Atasayar G et al.
Association of MDR1, CYP2D6, and CYP2C19 gene polymorphisms with prophylactic migraine treatment response.</td><td>3</td><td>152 migraine patients received amitriptyline prophylaxis for a minimum of 2 months. Treatment started with the minimal effective dose and the dose was increased up to the maximum effective dose according to treatment response and side effects. Only patients receiving amitriptyline monotherapy for prophylaxis and indicating no missed amitriptyline doses were evaluated.
Treatment response was defined as a decrease in the headache frequency during the preceding month with at least 50%.</td><td>Author’s conclusion:
”There were no significant correlations between the treatment responses to amitriptyline, propranolol, and valproic acid</td></tr>
</table>

<table>
<tr>
<td>J Neurol Sci 2016;366:149-154. PubMed PMID: 27288795.

ref. 2, continuation</td>
<td>IM: AA</td>
<td>44% of patients responded to treatment.
Relevant co-medication was not excluded.

Genotyping:
- 121x NM
- 31x IM

Results:
<table>
<tr><td colspan="2">Percentage of responders compared to NM (44% responders):</td></tr>
<tr><td>IM</td><td>NS</td></tr>
</table>

NB: Genotyping was for *2, *3 and *4. These are the most important gene variants in this Turkish population. None of the patients had *3 or *4.</td>
<td>and the MDR1, CYP2D6 and CYP2C19 gene polymorphisms.”</td>
</tr>
<tr>
<td>ref. 3
De Vos A et al. Association between CYP2C19*17 and metabolism of amitriptyline, citalopram and clomipramine in Dutch hospitalized patients. Pharmacogenomics J 2011;11:359-67. PubMed PMID: 20531370.</td>
<td>3

IM: AA

PM: A

UM: A</td>
<td>Routine therapeutic drug monitoring was performed for 150 patients on amitriptyline. Blood samples were drawn 12-16 h post-medication. The amitriptyline dose was known in 86 patients. The dose varied from 25-300 mg/day, with an average of 108 mg/day.
Relevant co-medication was not excluded.

Genotyping:
- 105x NM (60x *1/*1, 45x *1/*17) (59 with known dose)
- 8x UM (5 with known dose)
- 32x IM (21x *1/*2, 11x *2/*17) (19 with known dose)
- 5x PM (3 with known dose)

Results:
<table>
<tr><td colspan="7">Results compared to *1/*1 (or NM):</td></tr>
<tr><td></td><td>PM</td><td>*1/*2</td><td>*2/ *17</td><td>*1/ *17</td><td>UM</td><td>value for *1/*1 (or NM)</td></tr>
<tr><td rowspan="2">dose-corrected C_{ss} amitriptyline (ng/ml per mg)</td><td>x 1.36 (NS)</td><td>x 1.18 (NS)</td><td>x 1.00 (NS)</td><td>x 0.73 (NS)</td><td>x 0.55 (NS)</td><td>1.1</td></tr>
<tr><td>x 1.57 (NS)</td><td colspan="3">x 1.31 (NS)</td><td>x 0.63 (NS)</td><td>NM: 0.95</td></tr>
<tr><td rowspan="3">metabolic ratio (ami/nortriptyline)</td><td>x 3.14 (S)</td><td>x 1.21 (NS)</td><td>x 1.21 (NS)</td><td>x 1.00 (NS)</td><td>x 0.64 (NS)</td><td>1.4</td></tr>
<tr><td colspan="5">When only CYP2D6 NM were analysed, the difference was significant for both UM (x 0.50 (S)) and PM (x 2.31 (S)).</td><td>1.6</td></tr>
<tr><td>x 3.14 (S)</td><td colspan="3">x 1.21 (NS)</td><td>x 0.64 (NS)</td><td>NM: 1.40</td></tr>
<tr><td>% with subtherapeutic C_{ss} (ami+nor < 100 ng/ml)</td><td>NS for each CYP-2D6 phenotype</td><td>NS for each CYP-2D6 phenotype</td><td>NS for each CYP-2D6 phenotype</td><td>NS for each CYP-2D6 phenotype</td><td>NS for each CYP-2D6 phenotype</td><td>30%</td></tr>
<tr><td>% with suprathe-rapeutic C_{ss} (nortriptyline > 150</td><td>NS for each CYP-2D6 phe-noty-</td><td>NS for each CYP-2D6 phe-noty-</td><td>NS for each CYP-2D6 phe-noty-</td><td>NS for each CYP-2D6 phe-noty-</td><td>increase (S for CYP-2D6 NM, NS</td><td>0%</td></tr>
</table>
</td>
<td>Authors’ conclusion:
’This study confirms the increased activity of the CYP2C19*17 allele and shows increased metabolism of drugs that are metabolized by CYP-2C19, including amitriptyline and citalopram. However, the clinical relevance of CYP-2C19*17 is probably limited for amitriptyline, citalopram, and clomipramine.’</td>
</tr>
</table>

<table>
<tr>
<td>ref. 3, continuation</td>
<td></td>
<td>
<table>
<tr><td>ng/ml)</td><td>pe</td><td>pe</td><td>pe</td><td>pe</td><td>for CYP-2D6 IM and PM)</td><td></td></tr>
<tr><td rowspan="2">dose (mg/day)</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td rowspan="2">101</td></tr>
<tr><td colspan="5">There were also no significant differences after stratification for CYP2D6 phenotype (NM, PM, IM and UM) (NS).</td></tr>
<tr><td>C_{ss} amitriptyline (ng/ml)</td><td>x 1.84 (S)</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>77</td></tr>
<tr><td>C_{ss} nortriptyline (ng/ml)</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>71</td></tr>
<tr><td>C_{ss} ami+nortriptyline (ng/ml)</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>148</td></tr>
<tr><td rowspan="2">dose-corrected C_{ss} amitriptyline +nortriptyline, calculated from the mean values for corrected C_{ss} amitriptyline and the metabolic ratio (ng/ml per mg)</td><td>x 0.98 (NS)</td><td>x 1.09 (NS)</td><td>x 0.93 (NS)</td><td>x 0.73 (NS)</td><td>x 0.67 (NS)</td><td>1.89</td></tr>
<tr><td>x 1.13 (NS)</td><td colspan="2">x 1.21 (NS)</td><td></td><td>x 0.78 (NS)</td><td>NM: 1.63</td></tr>
</table>

NOTE: The relationship between amitriptyline concentration and dose was non-linear; a trend was observed for higher levels with higher dose, whereas the dose-corrected concentration value decreased with increased doses.

NOTE: Genotyping was for *2 and *17. These are the most important gene variants in this Dutch population.
</td>
<td>Estimated plasma concentration of amitriptyline+nortriptyline versus NM:
PM: 113%
IM: 121%
UM: 78%</td>
</tr>
<tr>
<td>ref. 4
van der Weide J et al.
Metabolic ratios of psychotropics as indication of cytochrome P450 2D6/ 2C19 genotype.
Ther Drug Monit 2005;27:478-83.
PubMed PMID: 16044105.</td>
<td>4</td>
<td>69 patients were treated with amitriptyline. Steady state serum trough concentrations were determined as part of routine therapeutic drug monitoring. Since De Vos 2011 included all patients treated with amitriptyline in this hospital since 1998 for which amitriptyline and nortriptyline serum concentrations and DNA were available, the patients in this article most probably are a subset of the patients in De Vos 2011.
Co-medication with CYP2C19 inhibitors and CYP2C19 inducers was excluded.

Genotyping:
- 52x NM
- 15x IM
- 2x PM</td>
<td>Authors' conclusion:
'According to these data, correlations exist between the log(MR) of venlafaxine, amitriptyline, and risperidone and the genotype of the CYP enzymes involved in their metabolism.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 4, continuation</td>
<td>PM: A
IM: A</td>
<td>Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td>metabolic ratio amitrityline/nor-triptyline</td><td>x 4.0 (S)</td><td>x 1.6 (S)</td><td>1.0</td></tr>
</table>
NOTE: Genotyping was for *2. These is the most important gene variant in this Dutch population.</td>
<td></td>
</tr>
<tr>
<td>ref. 5
Steimer W et al. Amitriptyline or not, that is the question: pharmacogenetic testing of CYP2D6 and CYP2C19 identifies patients with low or high risk for side effects in amitriptyline therapy.
Clin Chem 2005;51:376-85.
PubMed PMID: 15590749.</td>
<td>3</td>
<td>49 patients with at least medium-grade depressive disorder were treated amitriptyline for a period of 3 weeks. The amitriptyline dose was increased over the first 2 days and was then given at a fixed dose 75 mg twice daily. In five patients, the dose was lowered at the treating psychiatrist’s discretion, who was blinded for genotype and trough concentrations (75 mg/day, n = 1; 100 mg/day, n = 3; 125 mg/day, n =1).
Steady state had been reached at day 7. Mean trough concentrations of day 7 to day 21 were assessed. Depression was scored with the Hamilton Depression Scale (HAMD) and Clinical Global Impression Scale. Full response was defined as a value ≤ 8 and an improvement > 30% on the Hamilton Depression Scale (HAMD). Complete nonresponse was defined as a value ≥ 16 or an improvement < 30% on the HAMD.
Side effects were scored with the Dosage Record and Treatment Emergent Symptoms Scale (DOTES). DOTES consist of 30 items each rated on a 3-points scale and is organised in five clusters. DOTES scores ≥ 5 corresponded with above-average side effects.
88% of patients used psychotropic co-medication. Co-medication interfering with CYP2D6 or CYP2C19 metabolism was not excluded, but avoided whenever possible. 6 patients used possible CYP2C19-relevant co-medication (citalopram, diazepam, omeprazole), 13 patients possible CYP2D6-relevant co-medication (flupentixol, haloperidol, metoprolol, risperidone, sertraline, venlafaxine, yohimbine).

Genotyping:
- 30x NM (19x CYP2D6 NM, 11x CYP2D6 IM)
- 19x IM+PM (18x IM, 1x PM) (12x CYP2D6 NM, 6x CYP2D6 IM, 1x CYP2D6 UM)

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>CYP2D6 phenotype</td><td>IM+PM</td><td>value for NM</td></tr>
<tr><td rowspan="2">% of patients with full response</td><td>NM+UM</td><td>NS</td><td>52.6%</td></tr>
<tr><td>IM</td><td>NS</td><td>36.4%</td></tr>
<tr><td rowspan="2">% of patients with complete nonresponse</td><td>NM+UM</td><td>NS</td><td>12.1%</td></tr>
<tr><td>IM</td><td>NS</td><td>36.4%</td></tr>
<tr><td>% of patients with above-average side effects</td><td>all</td><td>NS</td><td>40.0%</td></tr>
<tr><td rowspan="3">total side effect score</td><td>NM+UM</td><td>trend for a decrease (p = 0.098; NS)</td><td>2.95</td></tr>
<tr><td>IM</td><td>NS</td><td>6.64</td></tr>
<tr><td colspan="3">For (IM+PM/CYP2D6 NM+UM) versus (NM/CYP2D6 NM+UM) versus (IM+ PM/CYP2D6 IM) versus (NM/CYP2D6</td></tr>
</table>
</td>
<td>Authors’ conclusion:
’Combined pharmacogenetic testing for CYP2D6 and CYP2C19 identifies patients with low risk for side effects in amitriptyline therapy and could possibly be used to individualize antidepressive regimens and reduce treatment cost. …. The lowest risk was observed for carriers of two functional CYP2D6 alleles combined with only one functional CYP-2C19 allele. …. We found no correlations between drug concentrations or genotypes and therapeutic response.’</td>
</tr>
</table>

ref. 5, continuation	IM+PM: A		IM), the effect was significant for the total side effect score and for the scores on the clusters mental side effects, anti-cholinergic/gastrointestinal symptoms and cardiovascular symptoms. There was a trend for the cluster neuromuscular symptoms (p = 0.554) and no significance for the cluster other symptoms.			
		C_{ss} nortriptyline (ng/ml)	NM+UM	trend for a decrease (p = 0.071; NS)	65.0	
			IM	NS	108.4	
		C_{ss} amitriptyline (ng/ml)	NM+UM	x 1.50 (S)	70.5	
			IM	NS	93.5	
		C_{ss} amitriptyline+ nortriptyline (ng/ml)	NM+UM	x 1.15 (NS)	134.7	
			IM	x 1.00 (NS)	201.9	
			Significance was not determined for IM+PM versus NM. There was a significant effect for (IM+PM/CYP2D6 NM+ UM) versus (NM/CYP2D6 NM+UM) versus (IM+ PM/CYP2D6 IM) versus (NM/CYP2D6 IM). However, for CYP-2D6 IM, the value was almost equal for IM+PM and NM. For CYP2D6 NM+ UM, the difference between IM+PM and NM was relatively small and thus not very likely to be significant.			
		amitriptyline/nortriptyline ratio	NM+UM	x 2.04 (S)	1.02	
			IM	x 1.23 (NS)	0.81	
			Significance was not determined for IM+PM versus NM. There was a significant effect for (IM+PM/CYP2D6 NM+ UM) versus (NM/CYP2D6 NM+UM) versus (IM+ PM/CYP2D6 IM) versus (NM/CYP2D6 IM). For CYP2D6 NM+ UM, the difference between IM+PM and NM was very likely to be significant, because of the significant increase in amitriptyline concentration and the trend for a significant decrease in the nortriptyline concentration for IM+PM compared to NM. For CYP2D6 IM, the difference between IM+PM and NM was unlikely to be significant, because both the increase in amitriptyline concentration and the decrease in nortriptyline concentration for IM+PM compared to NM were not significant.			
		NOTE: Nortriptyline concentration correlated with side effects, but amitriptyline concentration did not. However, the stronger influence on side effects of the phenotype of CYP2D6, which converts both amitriptyline and nortriptyline into inactive metabolites, than of the phenotype of CYP2C19, which converts amitriptyline into nortriptyline, suggests that the effect of nortriptyline concentration is due to its effect on the amitriptyline+nortriptyline concentration. The amitriptyline+nortriptyline concentration did not correlate with therapeutic response. NOTE: Genotyping was for *2, *3 and *4. These are the most				

<table>
<tr><td>ref. 5, continuation</td><td></td><td>important gene variants in this German population. Only *2 was detected in this patient group.</td><td></td></tr>
<tr><td>ref. 6
Steimer W et al.
Allele-specific change of concentration and functional gene dose for the prediction of steady-state serum concentrations of amitriptyline and nortriptyline in CYP2C19 and CYP-2D6 extensive and intermediate metabolizers.
Clin Chem 2004;50:1623-33.
PubMed PMID: 15205367.</td><td>3

IM: A

PM: A</td><td>Steady state serum concentrations of the 49 patients in Steimer 2005 were analysed.

Genotyping:
- 30x NM (19x CYP2D6 NM, 11x CYP2D6 IM)
- 18x IM (11x CYP2D6 NM, 6x CYP2D6 IM, 1x CYP2D6 UM)
- 1x PM (CYP2D6 NM)

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td>dose- and weight-corrected C_{ss} amitriptyline+nortriptyline</td><td>x 0.90 (NS)</td><td>x 1.06 (NS)</td><td>74.0 ng.kg/ml.mg</td></tr>
<tr><td>dose- and weight-corrected C_{ss} amitriptyline</td><td>NS</td><td>x 1.29 (S)</td><td>37.4 ng.kg/ml.mg</td></tr>
<tr><td rowspan="2">dose- and weight-corrected C_{ss} nortriptyline</td><td>NS</td><td>trend for a decrease (p = 0.059; NS)</td><td rowspan="2">37.0 ng.kg/ml.mg</td></tr>
<tr><td colspan="2">Multivariate linear regression showed a significant association of the CYP-2C19 phenotype with corrected nortriptyline concentration (S).</td></tr>
<tr><td>amitriptyline/nortriptyline ratio</td><td>NS</td><td>x 1.61 (S)</td><td>0.93</td></tr>
</table>
NOTE: Genotyping was for *2, *3 and *4. These are the most important gene variants in this German population. Only *2 was detected in this patient group.</td><td>Authors' conclusion:
'Eighteen CYP-2C19 heterozygotes (*1/*2) had higher amitriptyline and lower nortriptyline concentrations than 30 homozygotes (*1/*1). …. CYP2D6 but not CYP2C19 correlates with the sum of both concentrations used to guide amitriptyline therapy.'

Dose- and weight-corrected concentration of amitriptyline+nortriptyline versus NM:
PM: 90%
IM: 106%</td></tr>
<tr><td>ref. 7
Shimoda K et al.
The impact of CYP-2C19 and CYP2D6 genotypes on metabolism of amitriptyline in Japanese psychiatric patients.
J Clin Psychopharmacol 2002;22:371-8.
PubMed PMID: 12172336.</td><td>4

PM: A</td><td>50 patients were treated with a stable dose of amitriptyline for at least 2 weeks. Doses ranged from 25 to 225 mg/day (0.46-5.18 mg/kg body weight). Blood samples were drawn 9.5-16 hours after the evening dose.
Neuroleptics and barbiturates were excluded, because they affect amitriptyline metabolism. Benzodiapines were not excluded. None of the patients in the study took CYP-modulating agents.
Multiple regression analyses included age, gender, and the number of variant alleles of CYP2C19 and CYP2D6.

Genotyping:
- 24x NM
- 19x IM
- 7x PM

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td rowspan="2">dose- and weight-corrected C_{ss} amitriptyline</td><td>x 1.78 (S)</td><td>approximately x 1.11 (NS)</td><td rowspan="2">36.0 ng/ml per mg/kg</td></tr>
<tr><td colspan="2">Multiple regression analysis showed the number of CYP-2C19 variant alleles to be a</td></tr>
</table></td><td>Authors' conclusion:
'The genotype of CYP2C19 is one of the important determinants of the plasma concentrations of amitriptyline and the capacity to desmethylate amitriptyline. Mother compound amitriptyline is shunted via hydroxylation pathways from amitriptyline to E- and Z-hydroxy-amitriptyline in the subjects with homozygotes of mutated alleles of CYP2C19 in order to compensate for the decreased capacity to des-</td></tr>
</table>

<table>
<tr>
<td>ref. 7, continuation</td>
<td>IM: A</td>
<td>
<table>
<tr><td></td><td colspan="2">significant predictor for the corrected C_{ss} (S). The number of CYP2C19 variant alleles explained 12% of the variability in the logarithm of the corrected C_{ss}.</td><td></td></tr>
<tr><td>dose- and weight-corrected C_{ss} hydroxyamitriptyline</td><td>trend for an increase (p = 0.051; NS)</td><td>NS</td><td>9.5 ng/ml per mg/kg</td></tr>
<tr><td>metabolic ratio (ami/nortriptyline)</td><td>x 2.68 (S)</td><td>x 1.39 (S)</td><td>1.27</td></tr>
<tr><td></td><td colspan="2">Multiple regression analysis showed the number of CYP2C19 variant alleles to be a significant predictor for the metabolic ratio (S). The number of CYP2C19 variant alleles explained 37% of the variability in the logarithm of the metabolic ratio.</td><td></td></tr>
<tr><td>dose- and weight-corrected C_{ss} amitriptyline+nortriptyline, calculated from the mean values for C_{ss} amitriptyline and the metabolic ratio</td><td>x 1.29 (NS)</td><td>approximately x 0.97 (NS)</td><td>64.3 ng/ml per mg/kg</td></tr>
</table>
NOTE: Genotyping was for *2 and *3. These are the most important gene variants in this Japanese population.
</td>
<td>methylate amitriptyline.’

Estimated dose- and weight-corrected concentration of amitriptyline+nortriptyline versus NM:
PM: 129%
IM: 97%</td>
</tr>
<tr>
<td>ref. 8
Jiang ZP et al.
The role of CYP2C19 in amitriptyline N-demethylation in Chinese subjects.
Eur J Clin Pharmacol
2002;58:109-13.
PubMed PMID: 12012142.</td>
<td>PM: A
IM: AA</td>
<td>12 healthy volunteers, selected for their genotype, received a single dose of amitriptyline 50 mg.
Co-medication, smoking, alcohol and grapefruit juice were excluded.

Genotyping:
- 4x NM
- 2x IM
- 6x PM

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td>AUC amitriptyline+nortriptyline</td><td>x 1.07 (NS)</td><td>x 0.92 (NS)</td><td>2339 ng.h/ml</td></tr>
<tr><td>AUC amitriptyline</td><td>x 1.39 (S for PM versus NM+IM)</td><td>NS</td><td>1593 ng.h/ml</td></tr>
<tr><td>AUC nortriptyline</td><td>x 0.39 (S for PM versus NM+IM)</td><td>NS</td><td>746 ng.h/ml</td></tr>
<tr><td>metabolic ratio (ami/nortriptyline)</td><td>x 3.51 (S for PM versus NM+IM)</td><td>NS</td><td>2.17</td></tr>
</table>
NOTE: Genotyping was for *2 and *3. These are the most important gene variants in this Chinese population.
</td>
<td>Authors’ conclusion:
’The genetic defects of CYP2C19 have a significant effect on amitriptyline pharmacokinetics, and CYP2C19 plays an important role in N-demethylation of amitriptyline in vivo at a clinically therapeutic dose.’

AUC of amitriptyline+nortriptyline versus NM:
PM: 107%
IM: 92%</td>
</tr>
</table>

ref. 9 SmPC Amitriptyline HCl Apotex 29-08-17.	0 PM: A	Dose: Known poor metabolisers of CYP2D6 or CYP2C19 These patients can have a higher plasma concentration of amitriptyline and the active metabolite nortriptyline. Consider reducing the initial dose with 50%. Kinetics: The metabolism can be influenced by genetic polymorphisms (CYP2D6 and CYP2C19).	

Risk group	-

Comments:=

- Articles investigating amitriptyline and nortriptyline concentrations in other media than plasma (like hair) or post-mortem and cases without clinical effects of the variant genotype were not included in the risk analysis. In addition, an article only modelling data from another article was not included. These articles do not contribute enough to the evidence.
- Possible relationship between CYP2C19 polymorphisms and depression:
  - Jukić MM et al. Elevated CYP2C19 expression is associated with depressive symptoms and hippocampal homeostasis impairment. Mol Psychiatry 2017;22:1155-1163. PubMed PMID: 27895323.
    This publication is from the same group as Sim 2010.
    In a cohort of 3849 urban African-Americans of low economic status, the 123 CYP2C19*2/*2 subjects had a decrease in major depressive disorder prevalence compared to the other subjects with at least one active CYP-2C19 allele (23% versus 32%) (S). In addition, there was a trend for a lower Beck's Depression Inventory (BDI) score in the CYP2C19*2/*2 subjects compared to the other subjects (p = 0.074). However, the lifetime stress exposure was much larger in the African-American cohort compared with the previously analysed Swedish cohort (Sim 2010), thereby increasing the BDI score variability. After the most traumatized subjects (perceived stress scale score at higher quartile and above) were exempted from the analysis to better match the two samples, the BDI score reduction was significant (effect size = - 2.05 (-24.61%)) (S).
    In order to test whether the CYP2C19 genotype influences suicidality in patients with major depressive disorder, CYP2C19 genotype was tested as a predictor for suicide intent in 209 Western European suicide attempters with major depressive disorder. As there were only two CYP2C19*2/*2 allele carriers in the cohort, it was not possible to test whether this genotype affects Beck's suicide intent scale-objective circumstances (SIS-OS) score. However, in a complementary exploratory analysis, the SIS-OS score seemed to vary between different CYP2C19 genotypes with a decrease for *2/*2 versus *1/*1 versus *1/*2 versus *2/*17 versus *17/*17 versus *1/*17. Further analysis showed that SIS-OS score was not significantly affected by the presence of the CYP2C19*2 allele, whereas it was significantly increased in CYP2C19*17 allele carriers (119 versus 90 subjects, effect size = +1.36 (+25.69%)) (S). Since the score was lower for the 8 patients with genotype *17/*17 compared to the patients with genotype *1/*17, this significant effect seemed to be mainly driven by the *1/*17 genotype. The classification of the suicide attempters to severe (SIS-OS score at higher quartile and above) and non-severe, yielded a higher frequency of patients with *17 allele among severe suicide attempters (S).
    The authors conclude that the CYP2C19*2/*2 genotype associates with a phenotype more resilient to major depressive disorder and that the CYP2C19*17 allele may be a risk allele for suicidality in major depressive disorder. They indicate that a major limitation of the suicidality study is the absence of information regarding the individuals' drug treatment and their drug plasma levels. Therefore, it was not possible to determine whether the observed relationship was caused by endogenous or drug-metabolic CYP2C19-mediated effects.
  - Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium. A mega-analysis of genome-wide association studies for major depressive disorder. Mol Psychiatry 2013;18:497-511. PubMed PMID: 22472876.
    A mega-analysis of genome-wide association studies found no significant association between the risk of depression and CYP2C19.
  - Sim SC et al. Association between CYP2C19 polymorphism and depressive symptoms. Am J Med Genet B Neuropsychiatr Genet. 2010;153B:1160-6.
    In a group of 1472 Europeans older than 44 years (1017x NM (637x *1/*1, 380x *1/*17), 375x IM (290x *1/*2, 85x *2/*17), 35x PM (*2/*2), 45x UM), significantly lower depressive symptoms (measured on the Center of Epidemiologic Studies Depression (CES-D) scale) were found among PM patients than among *1/*1. There was only a difference among people younger than 73 years and among men. The effect size was in the same order of magnitude as that observed between non-users and users of antidepressants. The authors stated that CYP2C19 polymorphisms may have an effect on depressive symptoms in adult Europeans.
- Existing guideline:
  Hicks JK et al. Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. Clin Pharmacol Ther 2017;102:37-44. PubMed PMID: 27997040.

CPIC uses the same definitions of IM, PM and UM as we do. CPIC assigns *2/*17 and *3/*17 to the IM phenotype, because the currently available evidence indicates that the CYP2C19*17 increased function allele is unable to completely compensate for the CYP2C19 no function alleles, but indicates that this is a provisional classification, However, CPIC uses a different definition for NM (only *1/*1). CPIC created a new phenotype rapid metaboliser (RM) for *1/*17. CPIC also has nomenclature, but no recommendations for genotypes with very uncommon alleles with lower activity, e.g. *9 and *10. The summary below uses the KNMP definitions for NM, PM, IM and UM.
CPIC states that the usual amitriptyline starting dose may be used in CYP2C19 *1/*1 and IM. Although CYP2C19 IM would be expected to have a modest increase in the ratio of amitriptyline to nortriptyline plasma concentrations, the evidence does not indicate that CYP2C19 IM should receive an alternate dose. CPIC classifies this recommendation as strong (i.e. “the evidence is high quality and the desirable effects clearly outweigh the undesirable effects”).
CPIC states that patients taking amitriptyline who are CYP2C19 *1/*17 or UM may be at risk for having low plasma concentrations and an imbalance between parent drug and metabolites causing treatment failure and/or adverse events. However, CPIC states that the CYP2C19*17 allele did not alter the sum of amitriptyline plus nortriptyline plasma concentrations (De Vos 2011). Despite this, CPIC states that extrapolated pharmacokinetic data suggest that CYP2C19 *1/*17 or UM may need a dose increase (Stingl JC et al. Genetic variability of drug metabolizing enzymes: the dual impact on psychiatric therapy and regulation of brain function. Mol Psychiatry 2013;18:273-87). In addition, CPIC indicates that the CYP2C19*17 allele was associated with higher nortriptyline plasma concentrations, possibly increasing the risk of adverse events (De Vos 2011). However, nortriptyline is registered for use in depression and neuropathic pain itself. Therefore, it seems unlikely that an increased conversion of amitriptyline into nortriptyline would result in an increase in adverse events necessitating therapy adjustment. CPIC states that due to the need for further studies investigating the clinical importance of CYP2C19*17 regarding TCA metabolism and the possibility of altered concentrations, they recommend considering an alternative TCA or other drug not affected by CYP2C19. Due to limited available data, this recommendation is classified as optional (i.e. the desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action). CPIC states that if amitriptyline is administered to a CYP2C19 *1/*17 or UM, therapeutic drug monitoring is recommended.
CPIC states that CYP2C19 PM are expected to have a greater ratio of amitriptyline to nortriptyline plasma concentrations (Shimoda 2002). Although the total concentration of amitriptyline and nortriptyline may be unchanged for a CYP2C19 PM in certain instances, CPIC states that the elevated amitriptyline plasma concentrations may increase the chance of a patient experiencing side effects. CPIC recommends to consider a 50% reduction of the usual amitriptyline starting dose along with therapeutic drug monitoring (Stingl JC et al. Genetic variability of drug metabolizing enzymes: the dual impact on psychiatric therapy and regulation of brain function. Mol Psychiatry 2013;18:273-87). Although CPIC indicates that there is limited evidence demonstrating that a serotonergic/noradrenergic imbalance (i.e. amitriptyline/nortriptyline imbalance) influences outcomes and that therapeutic drug monitoring is based on the total concentration of amitriptyline and nortriptyline, this recommendation is classified as moderate (i.e. “there is a close or uncertain balance” as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects).
The therapeutic recommendations for amitriptyline are indicated below:

Dosing recommendations for amitriptyline for conditions requiring higher doses such as depression based on CYP2C19 phenotype[a]		
Phenotype	Therapeutic recommendation	Classification of recommendation
UM	Avoid amitriptyline use due to potential for sub-optimal response[e]. Consider alternative drug not metabolised by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. If amitriptyline is warranted, utilise therapeutic drug monitoring to guide dose adjustments.[b]	Optional[d]
*1/*17	Avoid amitriptyline use due to potential for sub-optimal response[e]. Consider alternative drug not metabolised by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. If amitriptyline is warranted, utilise therapeutic drug monitoring to guide dose adjustments.[b]	Optional[d]
*1/*1	Initiate therapy with recommended starting dose.[c]	Strong
IM	Initiate therapy with recommended starting dose.[c]	Strong
PM	Avoid amitriptyline use due to potential for sub-optimal response[e]. Consider alternative drug not metabolised by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. For amitriptyline, consider a 50% reduction of the recommended starting dose.[c] Utilise therapeutic drug monitoring to guide dose adjustments.[b]	Moderate

[a] Dosing recommendations only apply to higher initial doses of amitriptyline for treatment of conditions such as depression. For conditions at which lower initial doses are used, such as neuropathic pain, CPIC does recommend no dose modifications for PM or IM, because it is less likely that PM or IM will experience adverse effects due to supratherapeutic plasma concentrations of amitriptyline (Halling J et al. The CYP2D6 polymorphism in relation to the metabolism of amitriptyline and nortriptyline in the Faroese population. Br J Clin Pharmacol 2008;65:134-8). However, CPIC indicates that these patients should be monitored closely for side effects. In addition, if larger doses of TCA are warranted, CPIC recommends following the gene-based dosing guidelines in the table above.
For *1/*17 and UM, CPIC recommends considering an alternative agent, because pharmacokinetic data predict these patients to be at risk of failing amitriptyline therapy for neuropathic pain.
[b] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
[c] Patients may receive an initial low dose of a TCA, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
[d] The classification optional indicates that the desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action.
[e] Although the total concentration of amitriptyline and nortriptyline may be unchanged for a CYP2C19 ultra-rapid or poor metaboliser in certain instances, an imbalance between serotonergic and noradrenergic affect could influence clinical response or toxicities. There is limited evidence demonstrating that a serotonergic/noradrenergic imbalance influences outcomes, thus contributing to the classification of recommendations as optional or moderate.

As evidence linking CYP2C19 genotype with amitriptyline phenotype, CPIC mentions De Vos 2011, Koski 2006, Steimer 2005, Van der Weide 2005, Grasmäder 2004, Steimer 2004, Jiang 2002, Shimoda 2002, and Breyer-Pfaff 1992. All of these studies except for Koski 2006, Grasmäder 2004 and Breyer-Pfaff 1992 are included in our risk analysis. Koski 2006 was not included in our risk analysis because it concerns a post-mortem study. Grasmäder 2004 was not included, because the only data provided for amitriptyline separately, concerned a case without clinical effects of the variant genotype. Breyer-Pfaff 1992 was not included in our risk analysis, because it concerned a case without clinical effects of the variant genotype. CPIC indicates that these studies provide a high level of evidence for a decreased amitriptyline metabolism in PM and IM compared to *1/*1 (based on 8 references including Koski 2006 and Grasmäder 2004 for PM, and on 6 references including Koski 2006 for IM). In addition, De Vos 2011 provides a moderate level of evidence for an increased amitriptyline metabolism in UM compared to *1/*1 and Breyer-Pfaff 1992 a moderate level of evidence for a correlation of mephenytoin metabolism with amitriptyline metabolism.
CPIC also took other gene-based dosing recommendations in consideration, including the 2008 and 2011 publications of our dosing recommendations in Clinical Pharmacology and Therapeutics.
CPIC also provides therapeutic recommendations based on both CYP2D6 and CYP2C19 genotypes. For CYP2D6 UM and for CYP2D6 PM the therapeutic recommendations for the different CYP2C19 phenotypes are similar, reflecting the stronger influence of the CYP2D6 phenotype compared to the CYP2C19 phenotype. CPIC indicates that further studies are needed to develop moderate or strong dosing recommendations for TCAs when considering combined CYP2D6/CYP2C19 phenotypes. At the moment, insufficient data are available.
On 23-1-2019, there was not a more recent version of the recommendations present on the PharmGKB- and on the CPIC-site.

Date of literature search: 17 December 2018.

	Phenotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	PM	4 A	Yes	No	4 March 2019
	IM	4 A	Yes	No	
	UM	3 A	Yes	No	

Mechanism:
Amitriptyline is mainly converted by CYP2C19-mediated N-demethylation to the active metabolite nortriptyline. Both amitriptyline and nortriptyline are metabolised by CYP2D6 to 10-hydroxy metabolites, predominantly E-10-hydroxy metabolites. Amitriptyline is approximately three times as potent as E-10-OH-amitriptyline. Nortriptyline is approximately twice as potent as E-10-OH-nortriptyline.
N-oxidation and N-glucuronidation of amitriptyline also take place. Nortriptyline is converted by CYP2D6 and CYP-2C19 to the inactive metabolite didesmethylamitriptyline (desmethylnortriptyline).
Study results show an association between the sum of the concentrations of amitriptyline and nortriptyline with the efficacy of the therapy. The therapeutic range is 100-300 ng/ml and values higher than 400 ng/ml are considered to be toxic. An upper limit is indicated for the therapeutic range of nortriptyline (50-150 ng/ml), but not for the therapeutic range of amitriptyline (> 50 ng/ml). The Z-hydroxy metabolites can cause cardiotoxicity and plasma concentrations of Z-hydroxy nortriptyline or Z-hydroxy amitriptyline higher than 40 ng/ml are considered to be toxic.