CYP2D6: amitriptyline 1920/1921/1922

AMI = amitriptyline, Cl_{or} = oral clearance, C_{ss} = steady state concentration, EM = extensive metaboliser (gene dose 1.5-2.5) (normal CYP2D6 enzyme activity), IM = intermediate metaboliser (gene dose 0,5-1) (decreased CYP2D6 enzyme activity), MR = metabolic ratio, NS = non-significant, NORT = nortriptyline, PM = poor metaboliser (gene dose 0) (absent CYP2D6 enzyme activity), S = significant, SmPC = Summary of Product Characteristics, UM = ultra-rapid metaboliser (gene dose ≥ 3) (increased CYP2D6 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. Nortriptyline is converted by CYP2D6 and CYP2C19 to the inactive metabolite didesmethylamitriptyline (desmethylnortriptyline).
The therapeutic range is an amitriptyline+nortriptyline plasma concentration of 100-300 ng/ml and values higher than 400 ng/ml are considered to be toxic. 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.
Genetic variants in CYP2D6 can result in a decreased CYP2D6 enzyme activity (intermediate metabolisers (IM)), an absent CYP2D6 enzyme activity (poor metabolisers (PM)) or an increased CYP2D6 enzyme activity (ultra-rapid metabolisers (UM)).
All kinetic studies showed significant differences for patients with CYP2D6 gene variants (in the ratio between the 10-hydroxy metabolites and amitriptyline and nortriptyline, the ratio between amitriptyline and nortriptyline and/or the nortriptyline exposure). This indicates the presence of a CYP2D6/amitriptyline-interaction.

IM: One of the studies identified a correlation between IM and an increase in side effects (Steimer 2005, 17 IM). Therapy adjustment is therefore desirable (yes/yes-interaction). The weighted mean of the dose adjustment calculated on the basis of the increase in exposure of amitriptyline+nortriptyline is a dose reduction to 85% of the standard dose (median 71%, ranging per study from 71-93%). A dose reduction of 15% is actually too low to be clinically significant and thus, to be recommended. Because of the observed increase in side effects in Steimer 2005 and because of the higher median calculated dose reduction, the KNMP Pharmacogenetics Working Group decided to recommend the smallest clinically relevant dose reduction for IM, i.e. a reduction with 25% to 75% of the standard dose.

PM: There are insufficient data available for PM patients, but on theoretical grounds, the effect is expected to be more potent than that in IM. For this reason, the KNMP Pharmacogenetics Working Group decided that therapy adjustment is required for this gene-drug interaction (yes/yes-interaction). The weighted mean of the dose adjustment calculated on the basis of the increase in C_{ss} amitriptyline+nortriptyline is a dose reduction to 69% of the standard dose (median 68%, ranging per study from 68-69%). This was rounded up to 70% to be more achievable in clinical practice.

UM: One case found a correlation between UM and therapy failure (Bertilsson 1985). For this reason, the KNMP Pharmacogenetics Working Group decided that therapy adjustment is required for this gene-drug interaction (yes/yes-interaction). The weighted mean of the dose adjustment calculated on the basis of the change in C_{ss} amitriptyline+nortriptyline is a dose increase to 143% of the standard dose (median 122%, ranging per study from 60-184%) (results derived from two studies including a total of 3 UM). This was rounded off to 140% to be more achievable in clinical practice.
As hydroxy metabolites may have a cardiotoxic effect, an alternative is suggested as a second option.

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

Recommendation concerning pre-emptive genotyping, including justification of choices:
The Dutch Pharmacogenetics Working Group considers genotyping before starting amitriptyline to be potentially beneficial for the prevention of side effects and for drug efficacy. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the Dutch Pharmacogenetics Working Group recommends adhering to the gene-drug guideline.
The clinical implication of the gene-drug interaction scores 1 out of the maximum of 10 points (with pre-emptive genotyping considered to be potentially beneficial for scores ranging from 0 to 2 points) (see also the clinical implication score tables at the end of this risk analysis):
No severe clinical effects were observed in users of amitriptyline with a variant phenotype. The maximum severity code was C corresponding to CTCAE grade 2. This results in a score of 0 out of the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (only points for CTCAE grade ≥ 3).
The lack of a severe clinical effect also results in a score of 0 of the maximum of 3 points for the second and third criterion of the clinical implication score: the level of evidence supporting an associated clinical effect grade ≥ 3 and the number needed to genotype (NNG) in the Dutch population to prevent one clinical effect code ≥ D (grade ≥ 3).
The Summary of Product Characteristics (SmPC) of amitriptyline mentions the CYP2D6 PM phenotype, but does not mention this phenotype as a contra-indication and does not recommend pre-emptive genotyping. This results in 1 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (1 point for at least one genotype/phenotype mentioned in the SmPC, but not mentioned as a contra-indication and no recommendation to genotype).

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

Source	Code	Effect	Comments
ref. 1 Chaudhry M et al. Impact of CYP2D6 genotype on amitriptyline efficacy for the treatment of diabetic peripheral neuropathy: a pilot study. Pharmacogenomics 2017;18:433-443. PubMed PMID: 28350251.	3 IM: AA UM: AA	31 patients with painful diabetic peripheral neuropathy were treated with low-dose amitriptyline (10-100 mg/day; 25 mg/day in 64% of patients). During one week patients daily scored their satisfaction with the following six items on a scale of 0 to 10: the intensity of pain/burning in feet, blurred vision, constipation, drowsiness, dryness of the mouth and/or eyes, and difficulty urinating. Dissatisfaction was defined as a mean score higher than 5 (indicating dissatisfaction to prevail over satisfaction). Co-medication was not excluded. Bonferroni correction for 6 comparisons was applied (i.e. $p < 0.00833$ indicating significance). Genotyping: - 18x EM (11x gene dose 2, 7x gene dose 1.5) - 12x IM (10x gene dose 1, 2x gene dose 0.5) - 1x UM (gene dose 3) Results: Gene dose 0.5 versus gene dose 1.0 versus gene dose 1.5 versus gene dose 2 versus gene dose 3: - trend for more severe side effects in patients with lower gene doses (NS) - trend for a higher percentage of patients being dissatisfied with at least one side effect with lower gene doses (NS) - trend for a higher percentage of patients being dissatisfied with the pain intensity with lower gene doses (NS) - the UM patient did not report dissatisfaction with either pain intensity or any of the side effects on amitriptyline 25 mg/day. The patient concomitantly used the analgesic and CYP2C19 inhibitor indometacin. The study was a pilot study and too small to have a good probability for significant results. The authors calculated that 180 patients would be	Author's conclusion: "CYP2D6 genotype contributes to treatment outcome and may be useful for guiding drug therapy."

<table>
<tr><td>ref. 1, continuation</td><td></td><td>needed for each outcome measure to have a probability of at least 0.9 to be significant.

NB: Genotyping was performed by sequencing of the entire gene. Gene variants identified in this South-African population were *1, *2, *2M, *4, *5, *17, *29, *29+1SNP (2509G>T),*35, *41, *43, *45, *84, *1xN, *2xN and *4xN.</td><td></td></tr>
<tr><td>ref. 2
Ryu S et al.
A study on CYP2C19 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

IM: A</td><td>18 healthy volunteers, selected for their CYP2D6 and CYP2C19 genotype, received a single dose of amitriptyline 25 mg.
The subjects rated dry mouth and drowsiness on visual analogue scales predose 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 18 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:
- 6x *1/*1 (17% CYP2C19 EM, 83% CYP2C19 IM)
- 6x gene dose 1.5 + gene dose ≥ 2 (4x *1/*10, 1x *1/*10 with gene duplication, 1x *1xN/*5) (50% CYP2C19 EM, 50% CYP2C19 IM)
- 6x *10/*10 (67% CYP2C19 EM, 33% CYP2C19 IM)

Results:
Results compared to *1/*1:
<table>
<tr><td></td><td>*10/*10</td><td>gene dose 1.5 + gene dose ≥ 2</td><td>value for *1/*1</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</td><td>x 1.09
NS for *10/*10 versus (gene dose 1.5 + gene dose ≥ 2) versus *1/*1</td><td>x 0.94</td><td>265.60 ng.h/ml</td></tr>
<tr><td>AUC nortriptyline</td><td>x 1.89
NS for *10/*10 versus (gene dose 1.5 + gene dose ≥ 2) versus *1/*1 (S not determined)</td><td>x 1.21</td><td>171.16 ng.h/ml</td></tr>
<tr><td>AUC amitriptyline + AUC nortriptyline</td><td>x 1.40
NS for *10/*10 versus (gene dose 1.5 + gene dose ≥ 2) versus *1/*1 (S not determined)</td><td>x 1.05</td><td>436.76 ng.h/ml</td></tr>
<tr><td>AUC ratio 10-OH-amitriptyline/amitriptyline</td><td>x 0.33 (S)</td><td>x 0.74 (NS)</td><td>0.27</td></tr>
</table></td><td>Author’s conclusion: ”The extent of hydroxylation of amitriptyline or nortriptyline was significantly reduced in subjects carrying two CYP2D6 decreased functional alleles compared with those with no or one decreased functional allele. The gene variations of CYP2C19 and CYP2D6 did not change the pharmacodynamic effect.”

AUC amitriptyline + AUC nortriptyline versus *1/*1:
IM: 140%</td></tr>
</table>

<table>
<tr><td>ref. 2, continuation</td><td></td><td>
<table>
<tr><td>AUC ratio 10-OH-nortriptyline/nortriptyline</td><td>x 0.37 (S)</td><td>x 0.75 (NS)</td><td>1.9</td></tr>
<tr><td colspan="4">The authors indicated that the plasma concentrations in the subjects with gene duplication (gene dose ≥ 2) did not differ from the plasma concentrations in the subjects with gene dose 1.5.</td></tr>
</table>

NB: Genotyping of CYP2D6 was for *5, *10 and gene duplication, genotyping of CYP2C19 for *2, *3 and *17. These are the most important gene variants in this Korean population.
</td><td></td></tr>
<tr><td>ref. 3
Atasayar G et al. Association of MDR1, CYP2D6, and CYP-2C19 gene polymorphisms with prophylactic migraine treatment response. J Neurol Sci 2016;366:149-154. PubMed PMID: 27288795.</td><td>3

IM: AA
PM: AA</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%. 44% of patients responded to treatment. Relevant co-medication was not excluded.

Genotyping:
- 104x EM
- 41x IM
- 7x PM

Results:
<table>
<tr><td colspan="2">Percentage of responders compared to EM (45% responders):</td></tr>
<tr><td>IM</td><td>NS</td></tr>
<tr><td>PM</td><td>NS</td></tr>
</table>

NB: Genotyping was for *3, *4 and *6. These are the most important gene variants in this Turkish population. *6 was not detected in this population.</td><td>Author’s conclusion: ”There were no significant correlations between the treatment responses to amitriptyline, propranolol, and valproic acid and the MDR1, CYP2D6 and CYP2C19 gene polymorphisms.”</td></tr>
<tr><td>ref. 4
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.</td><td>3

IM: A

PM: A</td><td>Routine therapeutic drug monitoring was performed in 150 patients being treated with amitriptyline (71x EM (*1/*1), 58x IM (gene dose 1), 18x PM (gene dose 0), 3x UM (gene dose ≥ 3)). The amitriptyline dose was known in 86 patients (34x EM, 40x IM, 10x PM, 2x UM). Relevant co-medication was not excluded.

IM versus EM:
- Increase in the NORT C_{ss} by 29% (from 55 to 71 µg/L) (S)
- No significant difference in dose, AMI C_{ss}, and AMI+ NORT C_{ss}
- No difference in the dose-corrected AMI[b] C_{ss} (both 1.0 µg/L per mg/day) (NS)
- Decrease in the AMI/NORT MR by 18% (from 1.7 to 1.4) (S)
- Increase in the dose-corrected C_{ss} amitriptyline+nortriptyline, calculated from the mean values for dose-corrected C_{ss} amitriptyline and the metabolic ratio by 8% (from 1.59 to 1.71 µg/L per mg) (significance not determined)

PM versus EM:
- Increase in the NORT C_{ss} by 73% (from 55 to 95 µg/L) (S)
- No difference in dose and AMI C_{ss} (NS)</td><td>Authors’ conclusion: “Significant association of CYP2D6 genotype with amitriptyline metabolism was observed.”</td></tr>
</table>

ref. 4, continuation	UM: AA	- Increase in the AMI+NORT C_{ss} by 46% (from 129 to 188 µg/L) (S) - Increase in the dose-corrected AMI[b] C_{ss} by 30% (from 1.0 to 1.3 µg/L per mg/day) (NS) - Decrease in the AMI/NORT MR by 24% (from 1.7 to 1.3) (S) - Increase in the percentage of patients with NORT C_{ss} above the therapeutic range (> 150 µg/L) by 492% (from 2.8% to 16.7%) (S) - Increase in the dose-corrected C_{ss} amitriptyline+nortriptyline, calculated from the mean values for dose-corrected C_{ss} amitriptyline and the metabolic ratio by 45% (from 1.59 to 2.30 µg/L per mg) (significance not determined) UM versus EM: - No significant difference in dose, AMI C_{ss}, NORT C_{ss} and AMI+NORT C_{ss} - Decrease in the dose-corrected AMI[b] C_{ss} by 20% (from 1.0 to 0.8 µg/L per mg/day) (NS) - Increase in the AMI/NORT MR by 47% (from 1.7 to 2.5) (NS) - Decrease in the dose-corrected C_{ss} amitriptyline+nortriptyline, calculated from the mean values for dose-corrected C_{ss} amitriptyline and the metabolic ratio by 46% (from 1.59 to 0.86 µg/L per mg) (significance not determined)	Dose-corrected AMI + NORT plasma concentration versus EM: IM: 108% PM: 145% UM: 54%
ref. 5 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.	3 PM: A	Steady-state plasma concentrations were determined in 23 patients using amitriptyline (5-100 mg/day; median 25 mg/day) after exclusion of the two patients on the lowest doses (one PM on 5 mg/day and an EM[#] on 10 mg/day). Genotyping showed these 21 patients to be 10x EM (*1/*1), 7x IM (genotype not specified) and 4x PM (*4/*4). No co-medication with CYP2D6 inhibitors. PM versus EM[#]: - No difference in median dose - Increase in the AMI+NORT[b] C_{ss} by a median 48% and mean 69% (from median 4.4 to 6.5 nM/mg per day) (NS) - Increase in the AMI[b] C_{ss} by a median 62% and mean 28% (from median 2.4 to 3.89 nM/mg per day) (NS) - Increase in the NORT[b] C_{ss} by a median 129% and mean 121% (from median 1.49 to 3.42 nM/mg per day) (NS) - Increase in AMI/(E)-10-hydroxyAMI MR (S) - Increase in NORT/(E)-10-hydroxyNORT MR (S) - The AMI+NORT C_{ss} was higher than the therapeutic C_{ss} in 1 of the in total 5 PMs (454 nM versus 130-325 nM) Note: No genotyping for gene duplication was performed. Note: A high frequency of PM (15%) was observed in the Faroe Islands population investigated.	Dose-corrected AMI + NORT plasma concentration versus EM[#]: PM: 169%
ref. 6 Koski A et al. CYP2D6 and CYP2C19 genotypes and amitriptyline metabolite ratios in a series of medicolegal autopsies. Forensic Sci Int	3 IM: A	The effect of CYP2D6 genotype on amitriptyline metabolism was investigated in 195 post-mortem toxicology cases (13x PM, 60x IM, 108x EM, 14x UM). Co-medication with CYP2D6 inhibitors varied. IM versus EM: - Increase in AMI/(E)-10-hydroxyAMI MR (S) - Increase in NORT/(E)-10-hydroxyAMI MR (S) - Decrease in (E)-10-hydroxyNORT/(Z)-10-hydroxyNORT	Authors' conclusion: "Our study shows a concordance of AT metabolite patterns with CYP2D6 and CYP2C19 genotypes in the presence of confounding factors typical for post-

2006;158:177-83. **ref. 6, continuation**	 PM: A	MR (S) - Decrease in (Z)-10-hydroxyAMI/(Z)-10-hydroxyNORT MR (S) PM versus EM: - Increase in AMI/(E)-10-hydroxyAMI MR (S) - Increase in NORT/(E)-10-hydroxyAMI MR (S) - Decrease in (E)-10-hydroxyNORT/(Z)-10-hydroxyNORT MR (S) - Decrease in (Z)-10-hydroxyAMI/(Z)-10-hydroxyNORT MR (S) - Increase in NORT/(E)-10-hydroxyNORT MR (S) - Increase in NORT/(Z)-10-hydroxyAMI MR (S) - Decrease in (E)-10-hydroxyAMI/(Z)-10-hydroxyAMI MR (S) - Decrease in AMI/NORT MR (S) The cause of death in 103 cases was drug intoxication. Of these cases, 63 were primarily caused by AMI overdose, of whom 39 intended, 17 unintended and 7 not known. The unintended fatal intoxications were not associated with PM genotype (1x PM with a very low AMI concentration, 9x IM, 6x EM, 1x UM). Covariant analysis (CYP2D6, CYP2C19, age, gender) showed a dominant effect of CYP2D6 on AMI metabolism. Note: No genotyping for *41 was performed.	mortem material. This result demonstrates the feasibility of postmortem pharmacogenetic analysis and supports the dominant role of genes in drug metabolism.”
ref. 7 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.	3 IM: C	50 patients (32x EM, 17x IM, 1x UM) in a prospective, blinded study were given amitriptyline 150 mg/day for 3 weeks. In 5 patients, the psychiatrist adjusted the dose during the study period (up to 75 mg/day (n=1), 100 mg/day (n=3) and 125 mg/day (n=1)). Co-medication: 13x possible CYP2D6 inhibitors. IM versus EM+UM: - Increase in the percentage of patients with substantial side effects from 12.1% to 76.5% (S by 523%) Ditto for patients without CYP2D6-relevant co-medication: from 4.2% to 69.2% (S by 1548%) - Higher NORT C_{ss}: - From 49.0 to 101.2 µg/L for CYP2C19 IM+PM (S by 107%) - From 65.0 to 108.4 µg/L for CYP2C19 EM (S by 67%) - Higher (AMI + NORT) C_{ss} - From 154.8 to 202.0 µg/L for CYP2C19 IM+PM (S for the trend; by 30%) - From 134.7 to 201.9 µg/L for CYP2C19 EM (S for the trend; by 50%) - No difference in therapeutic response (NS) NORT concentrations correlated with side effects, but AMI concentrations 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 NORT + AMI concentrations did not correlate with therapeutic response.	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. Identification of genotypes associated with slightly reduced intermediate metabolism may be more important than currently anticipated.”
ref. 8 Steimer W et al. Allele-specific change	3	The same study as Steimer, 2005 (ref. 2) but further analysis of the pharmacokinetics. The number of patients was 3 for gene dose 0.5, 14 for gene dose 1.0, 11 for	Authors’ conclusion: “CYP2D6 but not CYP2C19 correlates

of concentration and functional gene dose for the prediction of steady-state serum concentrations of amitriptyline and nortriptyline in CYP-2C19 and CYP2D6 extensive and intermediate metabolizers. Clin Chem 2004;50:1623-33. **ref. 8, continuation**	IM: A	gene dose 1.5, 20 for gene dose 2.0 and 1 for gene dose 3.0. - Significant differences in NORT[a] C_{ss} when compared on the basis of gene dose (null allele = 0, partially functional allele = 0.5, functional allele = 1): - IM versus EM: - 0.5 versus 1.5: from 37.6 to 66.8 µg/L per kg/mg (S by 78%) - 0.5 versus 2.0: from 25.2 to 66.8 µg/L per kg/mg (S by 165%) - 1.0 versus 2.0: from 25.2 to 48.2 µg/L per kg/mg (S by 91%) - Low EM versus high EM: - 1.5 versus 2.0: from 25.2 to 37.6 µg/L per kg/mg (S by 49%) - The AMI+NORT C_{ss} is mainly affected by changes in NORT concentration as a result of CYP2D6 polymorphisms. The AMI+NORT[a] C_{ss} was: - Gene dose 0.5: 101.6 µg/L per kg/mg - Gene dose 1.0: 89.9 µg/L per kg/mg - IM: 92.0 µg/L per kg/mg - Gene dose 1.5: 75.1 µg/L per kg/mg - Gene dose 2.0: 59.8 µg/L per kg/mg - EM: 65.2 µg/L per kg/mg - Gene dose 3.0 (UM): 108.1 µg/L per kg/mg Note: The mean AMI+NORT C_{ss} and the mean NORT C_{ss} were consistent with the mean C_{ss} for patients with gene dose 1.5, so not with those of homozygous wild-type patients.	with the sum of both concentrations used to guide AT therapy." Dose- and body weight-corrected AMI + NORT plasma concentration versus EM: IM: 141% UM: 166%
ref. 9 Grasmader K et al. Impact of polymorphisms of cytochrome-P450 isoenzymes 2C9, 2C19 and 2D6 on plasma concentrations and clinical effects of antidepressants in a naturalistic clinical setting. Eur J Clin Pharmacol 2004;60:329-36.	3 UM: AA	136 patients on antidepressants, including 3 on amitriptyline (dose not known) were genotyped. Out of the 3 patients on AMI, 1 was a CYP2D6 UM and the other 2 were either CYP2D6 IM or EM. The mean dose-corrected C_{ss} of AMI + NORT was 0.61 ng/mL per mg of dosed AMI. For the UM, the corrected plasma concentration was 6% higher than the mean.	
ref. 10 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.	3 IM: A	50 patients received amitriptyline 25-225 mg/day (0.46-5.18 mg/kg per day) for ≥ 2 weeks. 8 patients had 0 mutant alleles (EM (genotype 1-1)), 32 patients had 1 mutant allele (29x EM (genotype 1-0.5) and 3x IM (genotype 1-0)), 10 patients had 2 mutant alleles (all IM, genotype 0.5-0.5 (n=8) or 0.5-0 (n=2)). IM versus EM: - Increase in the NORT/(E)-10-hydroxyNORT MR from 0.73 to 1.31 (NS by 79%) Multiple regression analysis showed that the number of CYP2D6 mutant alleles and gender together explained 17.7% of the variation in log (NORT/(E)-10-hydroxy-NORT). Note: No genotyping for gene duplication was performed.	

ref. 11 Mellstrom B et al. Amitriptyline metabolism: association with debrisoquin hydroxylation in nonsmokers. Clin Pharmacol Ther 1986;39:369-71.	3 IM: A PM: A	11 non-smokers received a 50-mg single dose of amitriptyline. Amitriptyline Cl_{or} showed a negative correlation with MR desibroquine/4-hydroxy-desibroquine MR in urine (S). NOTE: genotype unknown	Authors' conclusion: "Our data suggest that there may be a common regulation of the hydroxylation of debrisoquin and the oxidative metabolism of amitriptyline in nonsmokers."
ref. 12 Bertilsson L et al. Extremely rapid hydroxylation of debrisoquine: a case report with implication for treatment with nortriptyline and other tricyclic antidepressants. Ther Drug Monit 1985;7:478-80.	2 UM: C	Female patient on amitriptyline 50 mg three times daily. Plasma concentrations 3 and 5 weeks after initiation of treatment were 33 and 28 µg/L for AMI and 13 and <19 µg/L for NORT. After an initial short period of improvement in the depression, the patient had a relapse. The patient was previously treated with high-dose NORT (300-500 mg/day) and the NORT/10-hydroxyNORT MR was 0.13 at the time, suggestive of very rapid hydroxylation. The patient did not have severe anticholinergic side effects. NOTE: genotype unknown	Authors' conclusion: "Our patient developed low plasma levels of both AT and NT when she was treated with AT. There seem to be difficulties in optimizing the treatment of extremely rapid hydroxylators with all tricyclic antidepressants. In such cases it may be warranted to try a non-tricyclic antidepressant, which is not metabolized by the debrisoquine hydroxylase."
ref. 13 Baumann P et al. Amitriptyline pharmacokinetics and clinical response: II. Metabolic polymorphism assessed by hydroxylation of debrisoquine and mephenytoin. Int Clin Psychopharmacol 1986;1:102-12.	3 IM: A PM: A UM: A	16 patients (12x EM#, 4x PM) received amitriptyline (75 mg/day for 2 days, followed by 150 mg/day for 19 days). PM versus IM+EM+UM: - Lower MR (hydroxyAMI + hydroxyNORT)/(AMI + NORT) - 2 PMs had the highest AMI + NORT concentrations - PMs did not have excessive side effects - Clinical response could not be predicted on the basis of hydroxylation status or plasma concentrations of the active substances Correlations between desibroquine/hydroxydesibroquine MR in urine and AMI and metabolites in plasma: - Positive: AMI (S) and AMI+NORT (S) - Negative: hydroxyAMI/AMI (S), hydroxyNORT/NORT (S), (hydroxyAMI + hydroxyNORT)/(AMI + NORT) (S), (hydroxyAMI + hydroxyNORT)/AMI (S) NOTE: genotype unknown	Authors' conclusion: "The desibroquine-test appears to be a useful clinical tool for detecting in patients a genetic deficiency in the hydroxylation of AT-type drugs."
ref. 14 SmPC Amitriptyline HCl Apotex 29-08-17.	0 PM: A	<u>Dose</u>: 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%. <u>Kinetics</u>: The metabolism can be influenced by genetic polymorphisms (CYP2D6 and CYP2C19).	
ref. 15 SmPC Amitriptyline Hydrochloride Sandoz, USA, 17-07-14.	0	<u>Interactions</u>: The biochemical activity of the drug metabolizing isozyme cytochrome P450 2D6 (debrisoquin hydroxylase) is reduced in a subset of the caucasian population (about 7 to 10% of Caucasians are so called "poor metabolizers");	

ref. 15, continuation	PM: A	reliable estimates of the prevalence of reduced P450 2D6 isozyme activity among Asian, African and other populations are not yet available. Poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants (TCAs) when given usual doses. Depending on the fraction of drug metabolized by P450 2D6, the increase in plasma concentration may be small, or quite large (8 fold increase in plasma AUC of the TCA).	

[a]: Corrected for dose and body weight.
[b]: Corrected for dose.
[#]: Phenotyping and Halling et al., 2008 did not distinguish between IM, EM and UM. EM[#] is therefore equal to IM+EM+UM.

Risk group	IM with CYP2D6 inhibitors, UM with CYP2D6 inducers

Comments:

- Articles published after 2006 were only included if they either reported clinical effects or exposure of amitriptyline and nortriptyline in patients with different genotypes. The reason for this is that articles reporting metabolic ratios only supply insufficient additional information about the effect size of gene polymorphisms on amitriptyline therapy and about the magnitude of any dose adjustments needed.
- The risk analysis includes both genotyping and phenotyping studies. In order to make it easier to distinguish between these two types of studies, we have added the line “Note: genotype unknown” as the last line under phenotyping studies.
- 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 2016 Dec 20 [Epub ahead of print]. PubMed PMID: 27997040.
  CPIC uses the same definition for PM as we do. However, CPIC uses different definitions for EM (gene dose 1-2), IM (gene dose 0.5) and UM (gene dose > 2). In addition, CPIC changed the name of EM to normal metaboliser (NM). The summary below uses the KNMP definitions for EM, PM, IM and UM.
  CPIC states that the recommended starting dose of amitriptyline does not need dose adjustment for those with gene dose 1-2. In addition, CPIC states that a 25% reduction of the recommended dose may be considered for patients with a CYP2D6 gene dose of 0.5. As a reference for this percentage reduction they mention the 2011 publication of our dosing recommendations in Clinical Pharmacology and Therapeutics. However, this dosing recommendation is primarily based on patients with gene dose 1. In addition, we changed the percentage reduction in 2011 from 25% to 40%, based on the switch from using the sum of the plasma concentrations of amitriptyline and nortriptyline to using the plasma concentration of nortriptyline for dose calculations. Because patients with a CYP2D6 activity score of 1.0 are inconsistently categorised as intermediate or normal metabolisers in the literature, making these studies difficult to evaluate, CPIC classified the strength of the recommendation for gene dose 0.5 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).
  CPIC states that CYP2D6 ultra-rapid metabolisers + gene dose 2.5 have a higher probability of failing amitriptyline pharmacotherapy due to subtherapeutic plasma concentrations, and alternate agents are preferred.
  CPIC states that, if amitriptyline is warranted, there are insufficient data in the literature to calculate a starting dose for a patient with CYP2D6 ultra-rapid metaboliser or gene dose 2.5 status, and therapeutic drug monitoring is strongly recommended.
  Based on a nortriptyline study, CPIC indicates that adverse effects are more likely in CYP2D6 poor metabolisers due to elevated tricyclic plasma concentrations; therefore, alternate agents are preferred. If a tricyclic is warranted, CPIC recommends to consider a 50% reduction of the usual dose, and strongly recommends therapeutic drug monitoring.
  The therapeutic recommendations for amitriptyline are indicated below:

Dosing recommendations for amitriptyline for conditions requiring higher doses such as depression based on CYP2D6 phenotype[a]		
Phenotype	Therapeutic recommendation	Classification of recommendation
UM + gene dose 2.5	Avoid amitriptyline use due to potential lack of efficacy. Consider alternative drug not metabolised by CYP2D6. If amitriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolisers).[b] Utilise therapeutic drug monitoring to guide dose adjustments.	Strong

gene dose 1-2	Initiate therapy with recommended starting dose.[c]	Strong
gene dose 0.5	Consider a 25% reduction of recommended starting dose.[c] Utilise therapeutic drug monitoring to guide dose adjustments.[b]	Moderate
PM	Avoid tricyclic use due to potential for side effects. Consider alternative drug not metabolised by CYP2D6. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose.[c] Utilise therapeutic drug monitoring to guide dose adjustments.[b]	Strong

[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 recommends no dose modifications for PM or IM, because it is less likely that PM or gene dose 0.5 will experience adverse effects due to supratherapeutic plasma concentrations of amitriptyline. 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 UM+gene dose 2.5, CPIC recommends considering an alternative agent. Based on predicted and observed pharmacokinetic data in those with depression, CYP2D6 UM+gene dose 2.5 may be at an increased risk of failing amitriptyline therapy for neuropathic pain due to lower than expected drug concentrations (Dworkin RH et al. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007;13: 237-51).

[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 amitriptyline, 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.

As evidence linking CYP2D6 genotype with amitriptyline phenotype, CPIC mentions Peñas-Lledó 2013, de Vos 2011, Bijl 2008, Forget 2008, Halling 2008, Johnson 2006, Koski 2006, Steimer 2005, Steimer 2004, Shimoda 2002, Breyer-Pfaff 1992, Tacke 1992, Baumann 1986, Mellstrom 1986 and Balant-Gorgia 1982. All these studies, except for Balant-Gorgia 1982, Breyer-Pfaff 1992, Tacke 1992, the case reports Johnson 2006 and Forget 2008, Bijl 2008, and Peñas-Lledó 2013, are included in our risk analysis. In addition, our risk analysis includes the small study of Grasmader 2004, the case report of Bertilsson 1985, and three studies published in 2016 or 2017. CPIC indicates that these studies provide a high level of evidence for a decreased amitriptyline metabolism in PM compared to gene dose 1-2 (based on 8 references including Tacke 1992 and Balant-Gorgia 1982). In addition, CPIC indicates that these studies provide a high level of evidence for a correlation between the number/resulting function of CYP2D6 variant alleles and metabolism of amitriptyline (4 references). Contrary to this, CPIC indicates a weak level of evidence for the absence of a difference in metabolism of amitriptyline between carriers of only one CYP2D6 functional allele or carriers of decreased function alleles compared to carriers of two CYP2D6 normal function alleles (Shimoda 2002). CPIC indicates that these studies provide a moderate level of evidence for the requirement of a decreased dose of amitriptyline in PM compared to gene dose 1-2 (de Vos 2011) and for an increased risk of side effects in carriers of no function alleles compared to carriers of other alleles (Steimer 2005 and the case reports of Forget 2008 and Johnson 2006). In addition, CPIC indicates a moderate level of evidence for an association of PM with early discontinuation (within 28 days to 45 days after the start of the first prescription) of antidepressant therapy as compared to gene dose 1-2 (Peñas-Lledó 2013 and Bijl 2008), and for UM+gene dose 2.5 to have an increased risk for discontinuation of treatment and a decreased response (Peñas-Lledó 2013). Note: the majority of analysed patients in Peñas-Lledó 2013 and Bijl 2008 (54-55%) used another depressant than amitriptyline. Finally, CPIC indicates a moderate level of evidence for a correlation of desbrisoquine hydroxylation (Mellstrom 1986) and dextromethorphan metabolism (Breyer-Pfaff 1992) with amitriptyline metabolisme.

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+gene dose 2.5 and for CYP2D6 PM the therapeutic recommendations for the different CYP-2C19 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. Based on Steimer 2005, CPIC mentions that patients carrying at least one CYP2D6 no function allele and two CYP2C19 normal function alleles had an increased risk of experiencing side effects when administered amitriptyline. This would argue for a therapeutic recommendation also for patients with CYP2D6 gene dose 1, which is the predominant phenotype in this patient group.

On 22-5-2018, there was not a more recent version of the recommendations present on the PharmGKB- and on the CPIC-site.

Date of literature search: 30 April 2018.

	Phenotype	**Code**	**Gene-drug interaction**	**Action**	**Date**
Dutch Pharmaco-genetics Working	PM	3A	Yes	Yes	10 September 2018
	IM	3C	Yes	Yes	

Group decision	UM	3C	Yes	Yes	

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 and between nortriptyline concentrations and side effects. 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.

Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

Potentially beneficial	PGx testing for this gene-drug pair is potentially beneficial. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the DPWG recommends adhering to the gene-drug guideline	0-2 +
Beneficial	PGx testing for this gene-drug pair is beneficial. It is advised to genotype the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3-5 +
Essential	PGx testing for this gene-drug pair is essential for drug safety or efficacy. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection	6-10 +

Table 2: Criteria on which the attribution of Clinical Implication Score is based

Clinical Implication Score Criteria	**Possible Score**	**Given Score**
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)		
• CTCAE Grade 3 or 4 (clinical effect score D or E)	+	
• CTCAE Grade 5 (clinical effect score F)	++	
Level of evidence supporting the associated clinical effect grade ≥ 3		
• One study with level of evidence score ≥ 3	+	
• Two studies with level of evidence score ≥ 3	++	
• Three or more studies with level of evidence score ≥ 3	+++	
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3		
• 100 < NNG ≤ 1000	+	
• 10 < NNG ≤ 100	++	
• NNG ≤ 10	+++	
PGx information in the Summary of Product Characteristics (SmPC)		
• At least one genotype/phenotype mentioned	+	+
OR		
• Recommendation to genotype	++	
OR		
• At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	++	
Total Score:	10+	1+
Corresponding Clinical Implication Score:		Potentially beneficial