CYP2C9: phenytoin 1676 to 1682

*1 = no CYP2C9 gene variant, normal activity, *2 = CYP2C9 gene variant with decreased activity, *3 = CYP2C9 gene variant with strongly decreased activity, CI = confidence interval, C/D-ratio = concentration/dose ratio = dose-corrected plasma concentration, Cl_{or} = oral clearance, C_{ss} = steady-state plasma concentration, DRESS = drug reaction with eosinophilia and systemic symptoms, HR = hazard ratio, HSS = hypersensitivity syndrome, IM = = IM other = intermediate metaboliser, other genotype (decreased CYP2C9 enzyme activity due to the presence of one gene variant with decreased activity other than *2 or *3), MPE = maculopapular eruption, NM = normal metaboliser (*1/*1) (normal enzyme activity), NS = non-significant, $OR_{(corr)}$ = (corrected) odds ratio, p-HPPH = 5-(para-hydroxy-phenyl)-5-phenylhydantoin, PM = PM, other genotypes = combination of reduced-activity alleles including at least one allele other than *2 or *3, PM = PM other = poor metaboliser, other genotype (strongly decreased CYP2C9 enzyme activity due to the presence of two gene variants with decreased activity, of which at least one other than *2 or *3), S = significant, SJS = Stevens-Johnson syndrome, SmPC = Summary of Product Characteristics, TDM = therapeutic drug monitoring, TEN = toxic epidermal necrolysis, V_{max} = maximum elimination rate

Disclaimer: The KNMP Pharmacogenetics Working Group formulates optimal drug recommendations on the basis of the available evidence. If these optimal recommendations cannot be followed due to practical limitations, e.g. because therapeutic drug monitoring or lower doses are not available, healthcare professionals should consider the best available alternative.

Brief summary and justification of choices:
Phenytoin is predominantly metabolised by CYP2C9 (90%) and to a lesser extent by CYP2C19 (10%). Phenytoin exhibits non-linear pharmacokinetics. With chronic use, phenytoin induces CYP450 enzymes, primarily CYP2C9 and CYP2C19. So, phenytoin induces its own metabolism. However, because CYP2C9 and CYP2C19 are saturable at high phenytoin serum concentrations, small incremental doses may produce very substantial increases in serum concentrations, when these are in the upper range.
Polymorphisms in the CYP2C9 gene lead to an enzyme with reduced activity. This may increase plasma concentration at a certain dose, and may also lead to an increase in the incidence of side effects.

*1/*3, *2/*2, *2/*3: Human studies found an increased incidence of side effects for these genotypes (Fohner 2019, Su 2019, Wu 2018, Yampayon 2017, Tassaneeyakul 2016, Chung 2014, Depondt 2011, and Kesavan 2010). For *1/*3, this was confirmed by two case reports (McCluggage 2009 and Ninomiya 2000). This is why the KNMP Pharmacogenetics Working Group decided that a gene-drug interaction is present for these genotypes and that dose adjustment is required (yes/yes-interactions).

*3/*3: There were 3 cases with this genotype who developed side effects/toxicity on phenytoin (Jose 2008, Ramasamy 2007, and Brandolese 2001). Moreover, studies have found an increased incidence of side effects for *3/*3 and *1/*3 combined (Su 2019, Wu 2018, Yampayon 2017, Tassaneeyakul 2016 and Chung 2014), for *3/*3, *1/*3, and *2/*3 combined (Kesavan 2010), and for *3/*3, *1/*3, *2/*3, and *2/*2 combined (Fohner 2019). *1/*3, *2/*2, and *2/*3 lead to a smaller decrease in enzyme activity than *3/*3. This is why the KNMP Pharmacogenetics Working Group decided that this concerns a gene-drug interaction and that dose adjustment is required (yes/yes-interaction).

*1/*2: One study with 196 *1/*2 showed an increase in the switch to another anticonvulsant in the subset with seizures as indication (OR = 1.22), but this increase did not reach statistical significance in the full cohort (Fohner 2019). There was no increase in neurological side effects for *1/*2 in this study and other studies did not show an increased incidence of side effects for this genotype either (Kesavan 2010, Azzato 2010, and Hennessy 2009). There was one study that showed an increased incidence of side effects for *1/*2 and *1/*3 combined (Depondt 2011). Kinetic studies showed similar effects of *1/*2 and *1/*3 on AUC or plasma concentration (see also below). This is why the KNMP Pharmacogenetics Working Group decided that this concerns a gene-drug interaction and that dose adjustment is required (yes/yes-interaction).

PM: There was 1 case (*6/*6) in this genotype group with side effects on phenytoin (Kidd 2001). For this reason and analogous to *2/*2, *2/*3 and *3/*3, the KNMP Pharmacogenetics Working Group decided that this concerns a gene-drug interaction and that dose adjustment is required (yes/yes-interaction).

IM: One study among patients in this genotype group showed a 1.8-fold increase in dose and weight-corrected phenytoin trough concentrations (genotype *1/IVS8-109T) (Ortega-Vázquez 2016). For this reason and analogous to *1/*2 and *1/*3, the KNMP Pharmacogenetics Working Group decided that this concerns a

gene-drug interaction and that dose adjustment is required (yes/yes-interaction).

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

The justification of the dose recommendations is provided below per genotype/genotype group.

Justification of dose recommendations

Dose adjustments are calculated on the basis of (dose-corrected) AUC or steady-state plasma concentrations of phenytoin or on the basis of the dose needed to achieve plasma concentrations within the therapeutic range.

*1/*2 Calculations were based on 51 *1/*2 derived from 4 studies (Fohner 2019, Ortega-Vazquez 2015, Caraco 2001, and Aynacioglu 1999). The weighted mean of the calculated dose adjustment is a dose reduction to 73% of the normal dose (67%-107%; median 72%). This was translated to a reduction of the dose to 75%, in combination with monitoring of the plasma concentration (TDM).

*1/*3 Calculations were based on 103 *1/*3 derived from 8 studies (Fohner 2019, Yamamoto 2016, Hung 2012, Lee 2007, Hung 2004, Caraco 2001, Aynacioglu 1999, and Mamiya 1998). The weighted mean of the calculated dose adjustment is a dose reduction to 66% of the normal dose (51%-140%; median 69%). This was translated to a reduction of the dose to 70%, in combination with monitoring of the plasma concentration (TDM).

*2/*2 Calculations were based on 5 *2/*2 derived from 3 studies (Guevara 2017, Caraco 2001, and Aynacioglu 1999). The weighted mean of the calculated dose adjustment is a dose reduction to 56% of the normal dose (37%-63%; median 52%). This was translated to a reduction of the dose to 50%, in combination with monitoring of the plasma concentration (TDM).

*2/*3 There are only data for 1 healthy volunteer with the *2/*3 genotype (Caraco 2001). The calculated dose adjustment for this volunteer is a dose reduction to 37% of the normal dose. As a healthy volunteer with the *2/*2 genotype in this study had a similar AUC, this was translated to a reduction of the dose to 50%, in combination with monitoring of the plasma concentration (TDM).

*3/*3 Calculations were based on 4 *3/*3 derived from 4 studies (Hung 2012, Lee 2007, Kidd 2001, and Aynacioglu 1999). The weighted mean of the calculated dose adjustment is a dose reduction to 40% of the normal dose (22%-70%; median 33%). This was translated to a reduction of the dose to 40%, in combination with monitoring of the plasma concentration (TDM).

IM There are no data for IM other (combination of *1 with a reduced-activity allele other than *2 or *3). As the recommendation for IM other is analogous to the recommendation for *1/*2 and *1/*3 on theoretical grounds, this recommendation is also given here (reduction of the dose to 70-75% of the normal dose, in combination with monitoring of the plasma concentration (TDM)).

PM There are no data for PM other (combination of reduced-activity alleles, including at least one allele other than *2 or *3). As the recommendation for PM other is analogous to the recommendation for *2/*2, *2/*3 and *3/*3 on theoretical grounds, this recommendation is also given here (reduction of the dose to 40-50% of the normal dose, in combination with monitoring of the plasma concentration (TDM)).

Due to the longer half-life in patients with a gene variant, it will take longer to reach steady-state plasma concentrations. For this reason, the KNMP Pharmacogenetics Working Group recommends to measure plasma concentrations in patients with a gene variant 7-10 days after treatment start or dose adjustment, instead of the normal minimum period of 4-5 days.

Recommendation concerning pre-emptive genotyping, including justification of choices:

The KNMP Pharmacogenetics Working Group considers genotyping of patients before starting phenytoin maintenance therapy to be essential for drug safety. Genotyping must be performed before maintenance therapy has been initiated to guide dose selection.

The clinical implication of the gene-drug interaction scores 8 out of the maximum of 10 points (with pre-emptive genotyping considered to be essential for scores ranging from 6 to 10 points) (see below and the clinical implication score tables at the end of this risk analysis). The KNMP Pharmacogenetics Working Group restricted the genotyping recommendation to phenytoin maintenance therapy, because genotyping before start of phenytoin for acute indications, like status epilepticus and heart rhythm disorder, is both difficult to implement and not useful because no adjustment of the phenytoin loading dose is recommended in patients with a gene variant.

CYP2C9 gene variants have been shown to increase the risk of severe adverse events, including severe and possibly life-threatening cutaneous adverse events like SJS/TEN and DRESS. In one of the case-control studies showing *3 to increase SJS/TEN and DRESS risk, the adverse event was fatal in 9 out of the 48 SJS/TEN cases and 4 out of the 42 DRESS cases (code F corresponding to CTCAE grade 5) (Chung 2014). This results in the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for code F (CTCAE grade 5)).

Seven studies and three small meta-analyses (of 3, 3 and 4 case-control studies, respectively) showed an increased risk of adverse events with severity D-F (CTCAE grade 3-5). This results in the maximum of 3 points for the second criterion of the clinical implication score, the level of evidence supporting the associated clinical effect grade ≥ 3 (3 points for at least three publications with level of evidence score ≥ 3).

The largest study was performed in a USA patient cohort with *2 and *3 frequencies slightly smaller than those observed in the Dutch population (12.0% and 5.1% versus 14.2% and 9.2%) (Fohner 2019). This study showed an increase in the percentage of patients with neurological side effects (code D corresponding tot CTCAE grade 3) from 17% to 32% for *1/*3+*2/*2+*2/*3+*3/*3, comprising 11.8% of all patients (Fohner 2019). So, these genotypes caused additional severe adverse events in 1.77% of all patients (15% of 11.8%). This corresponds to the need to genotype 56 patients to prevent one additional adverse event with severity grade D. This results in 2 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) to prevent one clinical effect code ≥ D (grade ≥ 3) (two points for 10 < NNG ≤ 100).
The Summary of Product Characteristics (SmPC) contains a warning that *3 increases the risk of severe cutaneous adverse events and that carriers of *2 or *3 can have a higher risk of phenytoin toxicity, but neither mentions a CYP-2C9 gene variant or variant genotype as a contra-indication for phenytoin nor recommends 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 follows the KNMP definitions for IM and PM (IM other and PM other). The definitions of IM and PM used in the table below may therefore 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
Sukasem C et al. Genetic and clinical risk factors associated with phenytoin-induced cutaneous adverse drug reactions in Thai population. Pharmacoepidemiol Drug Saf 2020;29:565-74. PMID: 32134161.</td><td>3

*1/*3+ *3/*3: AA</td><td>87 cases with phenytoin-induced cutaneous adverse events (36 DRESS, 25 SJS/TEN, and 26 MPE) were compared to 69 phenytoin tolerant controls. Tolerant controls were defined as patients who had taken phenytoin for at least 3 months without cutaneous adverse events.
The median time to onset was 21 days (range 13-34 days) for SJS/TEN, 22 days (range 14-36 days) for DRESS, and 13 days (range 7-20 days) for MPE.
Phenytoin plasma concentration corrected for albumin level was supratherapeutic at the time of DRESS (25.3 µg/ml; n = 4) and SJS/TEN (26.9 µg/ml; n = 3), but not at the time of MPE (18.0 µg/ml; n = 2). Two weeks before the adverse event, it was also supratherapeutic for DRESS cases (28.3 µg/ml; n = 8), but within therapeutic range for SJS/TEN (10.3 µg/ml; n = 3) and MPE (17.0 µg/ml; n = 3) cases.
5.6% of SJS/TEN cases had TEN and 94.4% had SJS.
Relevant comedication was not excluded. Patients were included if the cutaneous adverse event was possibly, probably, or very probably caused by phenytoin according to Naranjo’s or ALDEN score. 28.6% of DRESS cases, 16.7% of SJS/TEN cases, and 16.0% of MPE cases used the CYP2C9 and CYP2C19 inhibitor omeprazole. Adjustment for omeprazole use in multivariate analysis was only performed for DRESS, not for SJS/TEN and MPE. Omeprazole was previously found to increase the risk of phenytoin-induced cutaneous adverse events, but was not found to increase the risk in this study.

Results:
<table>
<tr><td colspan="2">Percentage of *3 carriers in cases compared to tolerant controls (7.2%):</td></tr>
<tr><td>SJS/TEN</td><td>NS in univariate analysis and multivariate analysis</td></tr>
<tr><td></td><td>In multivariate analysis, there was a trend for an increased percentage of *3 carriers in cases compared to controls for the subgroup with Naranjo score ≥ 5 (probable and definite) or ALDEN score ≥ 6 (very probable) (p= 0.056) (NS).</td></tr>
<tr><td></td><td>The percentage of *1/*3 (7.2% in the controls) and of *3/*3 (0% in the controls) also did not differ between cases and tolerant controls in univariate analysis.</td></tr>
<tr><td></td><td>NS in univariate analysis and multivariate</td></tr>
</table>
</td><td>Author’s conclusion: ”CYP2C9*3 was almost reaching statistically associated with an increased risk of phenytoin-induced SJS/TEN (OR 4.800; 95% CI, 0.960-23.990; P = .056).”</td></tr>
</table>

<table>
<tr>
<td>ref. 1, continuation</td>
<td></td>
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<table>
<tr><td>DRESS</td><td>analysis</td></tr>
<tr><td></td><td>The percentage of *1/*3 (7.2% in the controls) and of *3/*3 (0% in the controls) also did not differ between cases and tolerant controls in univariate analysis.</td></tr>
<tr><td>MPE</td><td>NS in univariate analysis and multivariate analysis</td></tr>
<tr><td></td><td>The percentage of *1/*3 (7.2% in the controls) and of *3/*3 (0% in the controls) also did not differ between cases and tolerant controls in univariate analysis.</td></tr>
<tr><td>all cutaneous adverse events</td><td>NS in univariate analysis and multivariate analysis</td></tr>
<tr><td></td><td>The percentage of *1/*3 (7.2% in the controls) and of *3/*3 (0% in the controls) also did not differ between cases and tolerant controls in univariate analysis.</td></tr>
</table>

Note: Genotyping was for *3. This is the most important allele in this Thai population.
</td>
<td></td>
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<tr>
<td>ref. 2
Fohner AE et al. Assessing the clinical impact of CYP2C9 pharmacogenetic variation on phenytoin prescribing practice and patient response in an integrated health system. Pharmacogenet Genomics 2019;29:192-9. PMID: 31461080.</td>
<td>3</td>
<td>993 patients were treated with phenytoin (median starting dose 300 mg/day). CYP2C9 genotype was not known during treatment. Data on (dose-adjusted) blood concentrations were available for all patients (of whom 363 with a phenytoin starting dose of 300 mg/day, of whom 331 with reported data), data on switching to an alternative convulsant in 821 patients (of whom 159 with treatment of seizures as indication (as opposed to e.g. neuropathy or seizure prophylaxis following trauma or meningioma resection)), data on adherence and dose reduction in 732 patients (of whom 164 with treatment of seizures as indication), and data on side effects in 382 patients (of whom 232 with treatment of seizures as indication).
Neurological side effects were defined as neurological events (i.e., nystagmus, slurred speech, dizziness, somnolence) occurring within 100 days of first phenytoin dispensing.
A dose decrease was defined as a decrease in phenytoin dose from first phenytoin daily dose to last phenytoin daily dose within the first year of treatment, adjusted for whether the patient took concomitant anticonvulsants at any point during that year.
A switch to an alternative anticonvulsant was defined as filling a prescription for any alternative anticonvulsant in the first 100 days after the first dispensing of phenytoin and not subsequently filling another phenytoin prescription, with no prescription for an alternative anticonvulsant being filled in the 180 days prior to first phenytoin dispensing.
Poor patient adherence was defined as a gap of >30 days at any point between the end of supply and the date of a new prescription dispensing record in the first year of treatment.
The first therapeutic drug monitoring values were used as blood concentrations.
Relevant comedication was not excluded or adjusted for, with the exception of other anticonvulsants.
All regression analyses adjusted for age, sex and ethnicity. Regression for neurological side effects and for phenytoin adherence also adjusted for whether the patient was anticonvulsant-naïve. Regression for dose reduction also adjusted for whether the patient was anticonvulsant-naïve and for dual anticonvulsant therapy. Regression for supratherapeutic first blood concentration also adjusted for starting dose.

Genotyping:
all patients phenytoin starting dose of 300 mg/day and concen-</td>
<td>Author’s conclusion: ”CYP2C9 variation was associated with clinically-meaningful differences in clinician prescribing practice and patient response, with potential implications for healthcare utilization and treatment efficacy.”</td>
</tr>
</table>

<table>
<tr>
<td>ref. 2, continuation</td>
<td></td>
<td>
tration data reported
- 680x *1/*1
- 196x *1/*2
- 85x *1/*3
- 16x *2/*2
- 16x *2/*3 or *3/*3
- 231x *1/*1
- 63x *1/*2
- 25x *1/*3
- 12x *2/*2 or *2/*3

Results:
<table>
<tr><td colspan="4">Results compared to *1/*1:</td></tr>
<tr><td></td><td>*1/*3+*2/*2+ *2/*3+*3/*3</td><td>*1/*2</td><td>value for *1/*1</td></tr>
<tr><td>neurological side effect</td><td>HR = 2.40 (95% CI: 1.24-4.64) (S)</td><td>NS</td><td>17% of patients</td></tr>
<tr><td></td><td>Trend for an increase in the subset with seizures as indication (p = 0.07).</td><td>Also NS in the subset with seizures as indication.</td><td>22% of patients</td></tr>
<tr><td>phenytoin dose reduction in the first year</td><td>OR = 1.11 (95% CI: 1.02-1.22) (S).</td><td>NS</td><td></td></tr>
<tr><td></td><td>After exclusion of patients for whom it could not be confirmed that the first phenytoin prescription was indeed the first: trend for an increase (p = 0.07).</td><td>After exclusion of patients for whom it could not be confirmed that the first phenytoin prescription was indeed the first: OR = 2.07 (95% CI: 1.08-3.95) (S).</td><td></td></tr>
<tr><td></td><td>NS in the subset with seizures as indication.</td><td>Also NS in the subset with seizures as indication.</td><td></td></tr>
<tr><td>switch to another anticonvulsant in the first 100 days</td><td>NS</td><td>Trend for an increase (p = 0.08).</td><td></td></tr>
<tr><td></td><td>Also NS in the subset with seizures as indication.</td><td>OR = 1.22 (95% CI: 1.05-1.43) (S) in the subset with seizures as indication.</td><td></td></tr>
<tr><td>poor phenytoin adherence in the first year</td><td>OR = 1.95 (95% CI: 1.12-3.39) (S)</td><td>NS</td><td></td></tr>
<tr><td></td><td>NS in the subset with seizures as indication.</td><td>Also NS in the subset with seizures as indication.</td><td></td></tr>
<tr><td>first phenytoin</td><td>x 1.7 (NS)</td><td>x 1.4 (NS)</td><td>8.8</td></tr>
</table>
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</tr>
</table>

*1/*3+ *2/*2+ *2/*3+ *3/*3: D

*1/*2: D

<table>
<tr>
<td>ref. 2, continuation</td>
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<table>
<tr><td>blood concentration</td><td></td><td></td><td>µg/ml</td></tr>
<tr><td>supratherapeutic first phenytoin blood concentration</td><td>OR = 7.40 (95% CI: 3.09-17.70) (S)</td><td>OR = 4.08 (95% CI: 1.79-9.28) (S)</td><td></td></tr>
<tr><td>dose-adjusted first phenytoin blood concentration</td><td>increase with 21.3 pg/ml.mg (S)</td><td>increase with 8.6 pg/ml.mg (S)</td><td></td></tr>
<tr><td>first phenytoin blood concentration on a dose of 300 mg/day</td><td>*1/*3: x 1.84 (S)</td><td>x 1.44 (S)</td><td>8.8 µg/ml</td></tr>
</table>

Note: Genotyping was for variants mentioned in the PharmVar database. Only *2, *3, *5, *8, *11 and *12 were present in this patient group from the USA. Because of the low frequency of the *5, *8, *11 and *12 alleles and the likelihood that not all can be clustered in the same pheno-typic category, patients with these alleles were excluded from analysis.
</td>
<td>Blood concentration versus *1/*1:
*1/*2: 144%
*1/*3: 184%</td>
</tr>
<tr>
<td>ref. 3
Su SC et al.
HLA alleles and CYP2C9*3 as predictors of phenytoin hypersensitivity in East Asians.
Clin Pharmacol Ther 2019;105:476-85.
PMID: 30270535.</td>
<td>3

*1/*3+ *3/*3: E</td>
<td>
128 Taiwanese cases with phenytoin-induced severe cutaneous adverse events (65 SJS/TEN and 63 DRESS) and 107 patients with phenytoin-induced MPE were compared to 376 phenytoin tolerant controls. Tolerant controls were defined as patients who had taken phenytoin for more than 3 months without evidence for adverse events. In addition, a meta-analysis was performed of these Taiwanese data and data on 129 Thai cases (67 SJS/TEN and 62 DRESS) and 195 tolerant controls and data on 9 Japanese SJS/TEN cases and 94 tolerant controls.
The mean daily phenytoin dose was similar among cases and controls.
Relevant comedication, like CYP2C9 inhibitors, was not excluded. Patients were included if the ALDEN score for phenytoin causality was ≥4 (SJS/TEN) or the Naranjo score was ≥5 (DRESS).
A random effects model was used for the meta-analysis, but prospective registration of the protocol was not mentioned. The selection strategy was not mentioned, but the method of data assessment was transparent.
Quality of the included case-control studies was not assessed.
Selection bias analysis was not performed.

Results:
<table>
<tr><td colspan="4">Cutaneous adverse event risk for (*1/*3+*3/*3) compared to *1/*1:</td></tr>
<tr><td>cutaneous adverse event</td><td>OR</td><td>95% CI</td><td>*1/*3+*3/*3 frequency in the tolerant controls</td></tr>
<tr><td rowspan="3">severe cutaneous adverse event</td><td>17.87 (S)</td><td>8.35-38.24</td><td rowspan="3">2%</td></tr>
<tr><td colspan="2">30% of cases was *3 carrier.</td></tr>
<tr><td colspan="2">An association was also found in the meta-analysis of the Taiwanese, Thai and Japanese case-control study (OR = 7.12; 95% CI: 2.04-24.82) (S).</td></tr>
</table>
</td>
<td>Author’s conclusion: ”In addition to cytochrome P450 (CYP) 2C9*3, we found that HLA-B*13:01, HLA-B*15:02, and HLA-B*51:01 were significantly associated with phenytoin hypersensitivity with distinct phenotypic specificities.”</td>
</tr>
</table>

<table>
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<td>ref. 3, continuation</td>
<td></td>
<td>
<table>
<tr><td></td><td colspan="2">24% of cases and 4.2% of controls was *3 carrier.
Heterogeneity between the case-control studies was significant and high.</td></tr>
<tr><td>SJS/TEN</td><td>20.86 (S)</td><td>9.03-48.20</td></tr>
<tr><td></td><td colspan="2">34% of cases was *3 carrier.</td></tr>
<tr><td></td><td colspan="2">An association was also found in the meta-analysis of the Taiwanese, Thai and Japanese case-control studiy (OR = 8.60; 95% CI: 2.85-26.01) (S).
28% of cases and 4.2% of controls was *3 carrier.
Heterogeneity between the case-control studies was significant and moderate.</td></tr>
<tr><td>DRESS</td><td>16.95 (S)</td><td>6.93-41.47</td></tr>
<tr><td></td><td colspan="2">27% of cases was *3 carrier.</td></tr>
<tr><td></td><td colspan="2">No association was found in the meta-analysis of the Taiwanese and Thai case-control study (NS).
Heterogeneity between the case-control studies was significant and high.</td></tr>
<tr><td>MPE</td><td>4.20 (S)</td><td>1.66-10.63</td></tr>
<tr><td></td><td colspan="2">9% of cases was *3 carrier.</td></tr>
<tr><td>all</td><td>10.74 (S)</td><td>5.16-22.34</td></tr>
<tr><td></td><td colspan="2">21% of cases was *3 carrier.</td></tr>
</table>

Note 1: In the Taiwanese case-control study, ORs for *3 carriers were higher than for HLA-B*1502 (OR NS for DRESS and MPE and OR 3.01-6.52 for all cutaneous adverse events, severe cutaneous adverse events and SJS/TEN).

Note 2: 72% of the severe cutaneous adverse event cases, 74% of the SJS/TEN cases, 70% of the DRESS cases, and 22% of the controls was carrier of CYP2C9*3, HLA-B*1502, HLA-B*1301, and/or HLA-B*5101. Based on an incidence of severe cutaneous adverse events of 0.45%, the positive predictive value of the presence of one of these 4 alleles for development of a severe cutaneous event was calculated to be 1.4%, the negative predictive value 99.8%, and the number needed to genotype to prevent one severe cutaneous adverse event 310.
</td>
<td></td>
</tr>
<tr>
<td>ref. 4
Wu X et al. Association of CYP2C9*3 with phenytoin-induced Stevens-Johnson syndrome and toxic epidermal necrolysis: a systematic review and meta-analysis. J Clin Pharm Ther 2018;43:408-13. PMID: 29274302.</td>
<td>4</td>
<td>Meta-analysis of 4 case-control studies was performed. For tolerant controls, the meta-analysis included 3 studies with a total of 102 SJS/TEN cases and 322 controls. For population controls, the meta-analysis included 3 studies, of which 1 included 3 case-control studies (Chung 2014), with a total of 78 SJS/TEN cases and 4231 controls. All four studies were in Asian patients. With the exception of 2 small case-control substudies in Chung 2014 who could not be scored, all included studies scored 6 of the maximum of 9 points on the Newcastle-Ottawa Scale for study quality.
In two of the four studies, all of the patients in the case and control groups received other drugs concomitantly with phenytoin (Yampayon 2017 and Tassaneeyakul 2016), but none were considered to contribute to SJS/TEN in the study populations based on Naranjo and ALDEN algorithms.
3 of the 4 studies in this meta-analysis were also included in our risk analysis separately (Yampayon 2017, Tassanee-</td>
<td>Authors' conclusion: "A significant association between CYP2C9 *3 and phenytoin-induced SJS/TEN was identified, especially in a Thai population. CYP2C9*3 is thus a credible predictive genetic marker of phenytoin-induced SJS/TEN. Further multicentre studies and large prospective observational studies are, however, still required to determine the influence of CYP</td>
</tr>
</table>

<table>
<tr>
<td>ref. 4, continuation</td>
<td>*1/*3+
*3/*3: E</td>
<td>yakul 2016, and Chung 2014).
Meta-analyses were performed with a random-effects model in case of substantial heterogeneity between the studies and a fixed-effects model was chosen otherwise. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and data extraction was standardised.
Publication bias was evaluated with funnel plots (which were not shown).

Results:
<table>
<tr><td colspan="4">SJS/TEN risk for (*1/*3+*3/*3) compared to *1/*1:</td></tr>
<tr><td>controls</td><td>OR</td><td>95% CI</td><td>% *3 carriers in the controls</td></tr>
<tr><td rowspan="2">tolerant</td><td>8.93 (S)</td><td>2.63-30.36</td><td rowspan="2">4.7%</td></tr>
<tr><td colspan="2">32% of cases was *3 carrier.</td></tr>
<tr><td rowspan="2">population</td><td>8.88 (S)</td><td>5.01-15.74</td><td rowspan="2">5.7%</td></tr>
<tr><td colspan="2">36% of cases was *3 carrier.</td></tr>
<tr><td colspan="4">For tolerant controls, heterogeneity between the studies was significant and substantial. Heterogeneity disappeared after exclusion of the Taiwanese study of Chung 2014, leaving only the Thai studies of Yampayon 2017 and Tassaneeyakul 2016.
For population controls, heterogeneity between the studies was not significant.</td></tr>
<tr><td colspan="4">Funnel plots suggested publication bias, but it was difficult to determine publication bias because of the small number of included studies.</td></tr>
</table>
</td>
<td>2C*3 on blood levels of phenytoin and its metabolites, and their association with SJS/ TEN.”</td>
</tr>
<tr>
<td>ref. 5
Guevara N et al. Role of CYP2C9, CYP2C19 and EPHX polymorphism in the pharmacokinetic of phenytoin: a study on Uruguayan Caucasian subjects. Pharmaceuticals (Basel) 2017;10:73. PMID: 28820457.</td>
<td>3

*2/*2: A

*1/*2+
*1/*3: AA</td>
<td>50 patients were treated with phenytoin 1.85-8.89 mg/kg per day (mean 4.39 mg/kg per day).
For each patient, the mean of trough concentrations determined in at least three instances was used.
Hepatic or renal impairment was excluded. Relevant comedication was not excluded, although a remark in the discussion suggests that other CYP2C9 inducing antiepileptic drugs were.

Genotyping:
- 34x *1/*1
- 11x *1/*2
- 4x *1/*3
- 1x *2/*2

Results:
<table>
<tr><td colspan="4">Results compared to *1/*1:</td></tr>
<tr><td></td><td>*2/*2</td><td>*1/*2+*1/*3</td><td>value for *1/*1</td></tr>
<tr><td>dose- and weight-corrected phenytoin concentration</td><td>x 1.92 (S)</td><td>x 1.26 (NS)</td><td>1.63 µg.kg/ mg.mL</td></tr>
<tr><td>weight-corrected phenytoin dose</td><td>x 0.71 (NS)</td><td>x 0.90 (NS)</td><td>4.55 mg/kg</td></tr>
</table>
Note: Genotyping was performed for *2 and *3. These are the most important gene variants in this Uruguayan population.</td>
<td>Authors’ conclusion: “CYP2C9 was confirmed to be the main responsible enzyme for phenytoin biotransformation.”

Dose- and weight-corrected plasma concentration versus *1/*1:
*2/*2: 192%</td>
</tr>
</table>

<table>
<tr>
<td>ref. 6
Yampayon K et al. Influence of genetic and non-genetic factors on phenytoin-induced severe cutaneous adverse drug reactions. Eur J Clin Pharmacol 2017;73:855-65. PubMed PMID: 28391407.</td>
<td>3

*1/*3+ *3/*3: E</td>
<td>36 Thai cases with phenytoin-induced severe cutaneous adverse events (15 with Stevens-Johnson syndrome and 21 with DRESS or HSS) were compared to 100 phenytoin-tolerant controls. Phenytoin-tolerant controls were defined as patients without cutaneous adverse events after using phenytoin for at least 3 months. In addition, a population control of 250 Thai persons was used.
HSS was defined as DRESS except for the absolute eosinophil count being < 1500/µl.
Cases and controls were not matched and relevant comedication and comorbidities were not excluded.
Associations in bivariate analysis were also analysed by comparing cases to phenytoin-tolerant controls by multiple logistic regression.

Results:
<table>
<tr><td colspan="5">(*1/*3+*3/*3) compared to *1/*1:</td></tr>
<tr><td>cutaneous adverse event</td><td>controls</td><td>OR</td><td>95% CI</td><td>% *3 carriers in the controls</td></tr>
<tr><td rowspan="2">DRESS/ HSS</td><td>tolerant</td><td colspan="2">NS</td><td>6.0%</td></tr>
<tr><td>population</td><td colspan="2">NS</td><td>8.4%</td></tr>
<tr><td rowspan="3">SJS</td><td rowspan="3">tolerant</td><td>5.70 (S)</td><td>1.39-23.36</td><td rowspan="3">6.0%</td></tr>
<tr><td colspan="2">27% of cases was *3 carrier.</td></tr>
<tr><td colspan="2">Multiple logistic regression analysis showed *3 to be an independent risk factor for SJS: OR = 10.41 (95% CI: 2.06-55.42) (S). *3 and Chinese ancestry together explained 19% of the variation in phenytoin-induced SJS.</td></tr>
<tr><td>population</td><td>3.97 (S)</td><td>1.16-13.55</td><td>8.4%</td></tr>
</table>
Note 1: Genotyping was for *2 and *3, which are the most important gene variants in this Thai population. None of the cases and population controls and only 1 of the tolerant controls was *2 carrier. No association of *2 with DRESS/ HSS and SJS was found.

Note 2: In Europe, the estimated risks of SJS/TEN and DRESS/DHS in new users of phenytoin were 0.069% and 0.023-0.045%, respectively. The incidences in Asia were as high as 0.24% and 0.21% of phenytoin users for SJS/ TEN and DRESS, respectively.</td>
<td>Author’s conclusion: ”CYP2C9*3 and having Chinese ancestry were significant risk factors of SJS.”</td>
</tr>
<tr>
<td>ref. 7
Tassaneeyakul W et al. Associations between HLA class I and cytochrome P450 2C9 genetic polymorphisms and phenytoin-related severe</td>
<td>3</td>
<td>A case-control study including 60 cases of severe phenytoin-induced cutaneous side effects (39x SJS/TEN and 21x DRESS) compared to 92 controls who had used phenytoin for 6 months without developing cutaneous side effects. There was no difference in mean dose between the cases and controls (NS). Relevant co-medication was not excluded, but there were no differences in percentage of patients with CYP2C9 substrates/inhibitors between cases and controls (NS).</td>
<td>Authors’ conclusion: “The CYP2C9*3 variant was significantly associated with phenytoin-related SJS/TEN, but not DRESS.”</td>
</tr>
</table>

<table>
<tr>
<td>cutaneous adverse reactions in a Thai population. Pharmacogenet Genomics 2016;26:225-34. PubMed PMID: 26928377.</td>
<td>*1/*3+ *3/*3: E</td>
<td>Results:
<table><tr><td colspan="2">Effect of *3 on the risk of SJS/TEN and DRESS:</td></tr><tr><td>SJS/TEN</td><td>OR = 4.30 (95% CI: 1.41-13.09) (S)</td></tr><tr><td>DRESS</td><td>NS</td></tr><tr><td>SJS/TEN+DRESS</td><td>NS</td></tr></table>
Note: Alleles *2 and *3 were genotyped. *2 was not found in this Thai patient group.</td>
<td></td>
</tr>
<tr>
<td>ref. 8
Ortega-Vázquez A et al.
CYP2C9, CYP-2C19, ABCB1 genetic polymor-phisms and phenytoin plasma concentrations in Mexican-Mestizo patients with epilepsy. Pharmacogeno-mics J 2016;16:286-92. PubMed PMID: 26122019.</td>
<td>3

*1/*2: AA

IM+PM: A</td>
<td>64 patients were treated with phenytoin 100-600 mg/day (mean 306 mg/day).
Relevant co-medication was not excluded. 61% of the patients used relevant co-medication, but there was no significant association between plasma concentrations and CYP2C9 inducers or CYP2C9 inhibitors.

Genotyping:
*2:
- 61x *1/*1
- 3x *1/*2

IVS8-109A>T
- 42x AA
- 20x AT
- 2x TT

Results:
<table><tr><td colspan="2">Dose and weight-corrected phenytoin trough concen-trations compared to *1/*1 (3.0 µg.kg/mg.mL):</td></tr><tr><td>*1/*2</td><td>x 0.93 (NS)</td></tr><tr><td colspan="2">Note: No differences were found in the distribution of *1/*1 and *1/*2 over the groups with subtherapeutic, the-rapeutic and supratherapeutic phenytoin concentrations.</td></tr></table>
<table><tr><td colspan="5">IVS8-109TT versus IVS8-109AT versus IVS8-109AA:</td></tr><tr><td></td><td>TT</td><td>AT</td><td>AT + TT</td><td>value for AA</td></tr><tr><td>Dose and weight-corrected phenytoin trough concentrations</td><td>x 1.5 (NS)</td><td>x 1.8 (NS)</td><td>x 1.8 (S)</td><td>2.4 µg.kg/ mL.mg</td></tr><tr><td colspan="5">The frequency of the T-allele was 4.3x as high in the supratherapeutic group (plasma concentration > 20 µg/ mL) than in the subtherapeutic group (plasma concen-tration < 10 µg/mL) (S).
Both IVS8-109TT patients had supratherapeutic plasma concentrations (> 20 µg/mL).</td></tr></table>
Note 1: Genotyping was performed for *2, *3 and IVS8-109A>T. *3 was not found in this Mexican Mestizo popu-lation.

Note 2: The authors stated that the effect of IVS8-109A>T may be caused by linkage with other polymorphisms in the flanking region, exon 1 and intron 1. Kinetic studies inves-tigating the effect of the polymorphism on losartan give contradictory results. The results for phenytoin have there-fore not been included in the calculation of the dose correc-tion for IM and PM.</td>
<td>Authors' conclusion: "In contrast, we did not find an associa-tion between CYP2C9 (*2, *3) and CYP2C19 (*2, *3) alleles and PHT plasma concen-tration"

Plasma concentration versus *1/*1:
*1/*2: 93%</td>
</tr>
<tr>
<td>ref. 9
Yamamoto Y et al. Individualized phenytoin therapy for Japanese pediatric patients</td>
<td>3</td>
<td>Paediatric patients were treated with phenytoin for a period of 1 year. 17 patients received initial doses based on their CYP2C9 and CYP2C19 genotypes (mean 6.2 mg/kg per day for CYP2C9 *1/*1 and mean 3.0 mg/kg per day for CYP2C9 *1/*3) and a total of 139 patients received the conventional initial dose (mean 4.6 mg/kg per day). The</td>
<td>Authors' conclusion: "These findings sug-gest that genotyping can help to estimate the optimum target dose of phenytoin and</td>
</tr>
</table>

<table>
<tr>
<td>with epilepsy based on CYP2C9 and CYP2C19 genotypes.
Ther Drug Monit 2015;37:229-35.
PubMed PMID: 25162219.

ref. 9, continuation</td>
<td>*1/*3: A</td>
<td>dose was further titrated to TDM.
The dose requirement was determined for 170 patients.
Relevant co-medication was not excluded and the distribution of the CYP2C9 inducers phenobarbital and carbamazepine differed between the genotype groups and between the genotype-based and conventional therapy groups.
Plasma concentrations were determined 2-5 hours after dosing.
The dose requirement was defined as the dose that yielded steady-state plasma concentrations of 15-20 µg/mL without leading to dose-related side effects.
Only negative effects with a total score ≥ 5 on the Naranjo Adverse Drug Reaction (ADR) Probability Scale were considered to be drug related and therefore side effects.
Hazard ratios were corrected for age, sex and usage of other anti-epileptic agents.

Genotyping
Genotype-guided group (n = 17)
- 15x *1/*1
- 2x *1/*3
Dosing group (n = 170)
- 157x *1/*1
- 13x *1/*3

Results:
<table>
<tr><td colspan="3">Genotype-based initial dose versus conventional initial dose:</td></tr>
<tr><td></td><td></td><td>Value for conventional initial dose</td></tr>
<tr><td>Treatment discontinuation rate due to ineffectiveness or side effects.</td><td>Trend (NS; HR_{corr} = 0.37; p = 0.074)</td><td>33.1%</td></tr>
<tr><td>Time to treatment discontinuation</td><td>NS</td><td>294 days</td></tr>
</table>
<table>
<tr><td colspan="3">*1/*3 versus *1/*1:</td></tr>
<tr><td colspan="2"></td><td>value for *1/*1</td></tr>
<tr><td rowspan="3">Required phenytoin daily dose</td><td>x 0.72 (S)</td><td rowspan="3">7.3 mg/kg</td></tr>
<tr><td>The effect was also significant after correction for comedication other than sultiame in multiple regression analysis (S)</td></tr>
<tr><td>Body weight, CYP2C9 and CYP2C19 genotype and sultiame use together predicted 74% of the variability in dose requirement.</td></tr>
<tr><td>Peak plasma concentration</td><td>x 1.01 (NS)</td><td>17.2 µg/mL</td></tr>
</table>
Note: Alleles *2 and *3 were genotyped. *2 was not found in this Japanese population.</td>
<td>Dose versus *1/*1: *1/*3: 72%</td>
</tr>
<tr>
<td>ref. 10
Chung WH et al. Genetic variants associated with</td>
<td>3</td>
<td>A case-control study including 168 cases of phenytoin-induced cutaneous side effects (48x SJS/TEN, 42x DRESS and 78x maculopapular exanthema) compared to 130 patients who had used phenytoin for more than 3 months</td>
<td>Authors’ conclusion: ‘This study identified CYP2C variants, including CYP2C9*3,</td>
</tr>
</table>

<table>
<tr>
<td>phenytoin-related severe cutaneous adverse reactions. JAMA 2014;312:525-34. PubMed PMID: 25096692.

ref. 10, continuation</td>
<td>*1/*3+ *3/*3: F</td>
<td>without developing cutaneous side effects or with the general population (n = 412). Relevant co-medication was not excluded. A total of 13 of the cases died, including 9 SJS/ TEN cases and 4 DRESS cases. Doses were determined in 40 cases, plasma concentrations before treatment discontinuation in 9 cases (including 2x *1/*1).
Moreover, a meta-analysis was performed for SJS, TEN and DRESS of this and smaller case-control studies in Asian patients, all with the general population as the control group. For SJS/TEN, the meta-analysis included 3 studies involving a total of 61 cases and 3655 controls. For DRESS, the meta-analysis included 2 studies involving a total of 44 cases and 786 controls.
ORs in the meta-analysis were calculated using a random-effects model, but prospective registration of the protocol was not mentioned. The selection strategy was not mentioned, but the method of data assessment was transparent. Quality of the included case-control studies was not assessed.
Selection bias analysis was not performed.

Results:
Case-control study:
<table>
<tr><td colspan="5">(*1/*3 + *3/*3) versus *1/*1:</td></tr>
<tr><td></td><td colspan="2">Phenytoin control</td><td colspan="2">Population control</td></tr>
<tr><td></td><td>OR</td><td>95% CI</td><td>OR</td><td>95% CI</td></tr>
<tr><td>SJS/TEN</td><td>30 (S)
42% of the cases had *3.</td><td>8.4-109</td><td>14.0 (S)</td><td>6.8-29.0</td></tr>
<tr><td>DRESS</td><td>19 (S)
31% of the cases had *3.</td><td>5.1-71</td><td>8.8 (S)</td><td>4.0-19.4</td></tr>
<tr><td>Maculopapular exanthema</td><td>5.5 (S)</td><td>1.5-21</td><td>2.6 (S)</td><td>1.1-5.9</td></tr>
<tr><td></td><td colspan="3"></td><td>Value for *1/*1</td></tr>
<tr><td>Phenytoin peak plasma concentration</td><td colspan="3">x 2.2 (S)
The values for *1/*1 were in line with the values for the controls.</td><td>11 µg/mL</td></tr>
<tr><td>Daily dose</td><td colspan="3">NS</td><td>304 mg</td></tr>
</table>
Meta-analysis:
<table>
<tr><td colspan="3">(*1/*3 + *3/*3) versus *1/*1:</td></tr>
<tr><td></td><td>OR</td><td>95% CI</td></tr>
<tr><td>SJS/TEN</td><td>12.0 (S)</td><td>6.4-22.3</td></tr>
<tr><td>DRESS</td><td>9.2 (S)</td><td>4.3-19.8</td></tr>
<tr><td>SJS, TEN or DRESS</td><td>10.6 (S)</td><td>6.2-18.2</td></tr>
<tr><td colspan="3">There was no study heterogeneity.</td></tr>
</table>
</td>
<td>known to reduce drug clearance, as important genetic factors associated with phenytoin-related severe cutaneous adverse reactions.’</td>
</tr>
<tr>
<td>ref. 11
Hung CC et al. Effects of polymorphisms in six candidate genes on phenytoin maintenance therapy in Han Chinese patients. Pharmacogenomics 2012;13:1339-49.</td>
<td>4</td>
<td>269 patients received maintenance therapy with phenytoin (mean 315.48 mg/day) as their only anti-epileptic agent. Relevant co-medication was excluded.

Genotyping:
- 252x *1/*1
- 16x *1/*3
- 1x *3/*3
<table>
<tr><td colspan="4">*3/*3 versus *1/*3 versus *1/*1:</td></tr>
<tr><td></td><td>*3/*3</td><td>*1/*3</td><td>Value for *1/*1</td></tr>
</table>
</td>
<td>Authors’ conclusion: ‘In a multivariate analysis, variants in CYP2C9, CYP2C19, SCN1A, and ABCB1 genes were significantly associated with concentration-dose ratios of phenytoin under adjustment of age, gender and epilepsy classifications.’</td>
</tr>
</table>

<table>
<tr>
<td>PubMed PMID: 22966884.

ref. 11, continuation</td>
<td>*1/*3: A
*3/*3: AA</td>
<td>
<table>
<tr><td>Dose-corrected phenytoin plasma concentration</td><td>x 2.32</td><td>x 1.93 (S)</td><td>34.8 day/mL</td></tr>
<tr><td>Daily dose</td><td>x 0.59</td><td>x 0.68 (S)</td><td>5.65 mg/kg</td></tr>
<tr><td>Phenytoin plasma concentration</td><td>x 1,06</td><td>x 1,06 (NS)</td><td>15.19 mg/L</td></tr>
<tr><td colspan="4">Results were similar after Bonferroni correction for multiple testing.</td></tr>
<tr><td colspan="4">Multivariate regression analysis, which corrected for epilepsy type, weight, age and sex, found an effect on dose-corrected plasma concentration for CYP2C9*3 (S).</td></tr>
</table>

Note: genotyping was performed for *3. This is the most important allele in this Han-Chinese population.
</td>
<td>Plasma concentration versus *1/*1:
*1/*3: 193%
*3/*3: 232%</td>
</tr>
<tr>
<td>ref. 12
Depondt C et al. A candidate gene study of antiepileptic drug tolerability and -efficacy identifies an association of CYP2C9 variants with phenytoin toxicity. Eur J Neurol 2011;18:1159-64. PubMed PMID: 21338443.</td>
<td>3

*1/*2+
*1/*3: D
*2/*2: D</td>
<td>137 patients were treated with phenytoin. Relevant co-medication was not excluded. Corrections were made for age, sex and multiple testing.

Genotyping:
- 86x *1/*1
- 43x *1/*2
- 6x *1/*3
- 2x *2/*2

Results:
<table>
<tr><td colspan="3">% of patients with side effects versus *1/*1 (26%):</td></tr>
<tr><td>*1/*2 + *1/*3</td><td>x 2.1</td><td rowspan="2">S for the trend *2/*2 versus (*1/*2 + *1/*3) versus *1/*1</td></tr>
<tr><td>*2/*2</td><td>x 2.0</td></tr>
</table>

Note: Alleles *2 and *3 were genotyped.
</td>
<td>Authors’ conclusion: ‘Our results confirm the role of CYP2C9 variants in phenytoin toxicity.’</td>
</tr>
<tr>
<td>ref. 13
Kesavan R et al. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on phenytoin-induced neurological toxicity in Indian epileptic patients. Eur J Clin Pharmacol 2010;66:689-96. PubMed PMID: 20390258.</td>
<td>3

*1/*2: AA

*1/*3: D

*2/*3: D</td>
<td>292 patients were treated with phenytoin for more than 2 months. Neurotoxicity was defined as occurrence of CNS-related side effects. Phenytoin plasma concentrations were higher in patients with neurotoxicity than in patients without neurotoxicity (23.0 and 9.1 µg/mL respectively) (S). Relevant co-medication was not excluded. ORs were corrected for dose.

Genotyping:
- 244x *1/*1
- 14x *1/*2
- 26x *1/*3
- 5x *2/*3
- 3x *3/*3

Results:
<table>
<tr><td colspan="3">Risk of neurotoxicity versus *1/*1 (neurotoxicity in 12% of the patients):</td></tr>
<tr><td>*1/*2</td><td colspan="2">Trend (NS; OR_{corr} = 3.5; 95% CI: 0.9 - 12.4)</td></tr>
<tr><td>*1/*3</td><td colspan="2">OR_{corr} = 15.3 (95% CI: 5.8 - 40.3) (S)</td></tr>
<tr><td>*2/*3</td><td colspan="2">100% of patients had neurotoxicity (NS, significance not determined)</td></tr>
<tr><td>*3/*3</td><td colspan="2">100% of patients had neurotoxicity (NS, significance not determined)</td></tr>
<tr><td></td><td></td><td>Value for *1</td></tr>
<tr><td>*2 (= *1/*2 +</td><td>x 3.1 (S)</td><td>15%</td></tr>
</table>
</td>
<td>Authors’ conclusion: “Our results show that CYP2C9 genetic polymorphisms (particularly the *3 allele) were associated with high risk of epileptic patients developing phenytoin-induced neurological toxicity.”</td>
</tr>
</table>

ref. 13, continuation	*1/*3+ *2/*3+ *3/*3: D	*2/*3)	
		*3 (= *1/*3 + *2/*3 + *3/*3)	x 4.8 (S)
		Of the patients with neurotoxicity, the percentage with severe toxicity increased with the number of CYP2C9 variant alleles (0% for *1/*1; 5% for *1/*2 + *1/*3 and 56% for *2/*3 + *3/*3) (NS, significance not determined). Note: Alleles *2 and *3 were genotyped.	
ref. 14 Azzato EM et al. Maternal EPHX1 polymorphisms and risk of phenytoin-induced congenital malformations. Pharmacogenet Genomics 2010;20:58-63. PubMed PMID: 19952982.	3 *2: AA *3: -	The ~135 mothers of ~152 children used phenytoin for epilepsy during pregnancy. None of the pregnancies were multiple pregnancies. Relevant co-medication was not excluded. ORs were corrected for maternal age, ethnic origin, education, number of cigarettes per day, maximum phenytoin daily dose, occurrence of epileptic seizures during pregnancy, use of phenytoin during the first trimester, continuous daily phenytoin usage throughout the pregnancy and phenobarbital usage. The study had insufficient power. The power was 25% for an OR of 2.5 and 9% for an OR of 1.5. Maternal genotype: *2: - ~139x *1/*1 - ~13x (*1/*2+*2/*2+*2/*3) *3: - ~149x *1/*1 - ~3x (*1/*3+*2/*3+*3/*3) Results: Risk of severe craniofacial abnormalities in the child versus (no *2) or (no *3) (abnormalities in ~12% of the children): *2: NS *3: n too small to calculate an OR	Authors' conclusion: "We did not observe any associations between maternal CYP2C9 *2 or *3 polymorphisms and presence of major craniofacial abnormalities in the child."
ref. 15 Hennessy S et al. CYP2C9, CYP2C19, and ABCB1 genotype and hospitalization for phenytoin toxicity. J Clin Pharmacol 2009;49:1483-7.	3 *1/*2: AA *1/*3: AA *2/*2: AA	Case-control study, 14 cases (1x *2/*2, 1x *1/*3, 4x *1/*2, 8x *1/*1), admitted to hospital as a result of phenytoin toxicity (side effects on the central nervous system). *1/*2 versus *1/*1: – No difference in the risk of hospital admission due to phenytoin toxicity. *1/*3 versus *1/*1: – Non-significant increase in the risk of hospital admission due to phenytoin toxicity. *2/*2 versus *1/*1: – Non-significant increase in the risk of hospital admission due to phenytoin toxicity.	Authors' conclusion: "Given the wide CIs associated with the ORs for other genotypes, this study certainly neither demonstrates nor excludes a potential role of the genes studied in the etiology of acute CNS toxicity of phenytoin."
ref. 16 McCluggage LK et al. Phenytoin toxicity due to genetic polymorphism. Neurocrit Care 2009;10:222-4.	2 *1/*3: D	A 53-year-old woman remained lethargic for 4 days and developed hyperglycaemia and fever after starting intravenous phenytoin (single dose of 1,000 mg, followed by 300 mg/day) for prevention of epileptic seizures following a traumatic brain injury. The plasma concentration of free phenytoin was 4.4 mg/L (target value 1-2 mg/L) and the woman was found to be CYP2C9 *1/*3 and CYP2C19 *1/*1. Apart from the genotype, there were no other explanations for the toxic plasma concentration. The half-life was 72 hours (normal value is 22 hours).	Authors' conclusion: "This case reveals the clinical significance of genetic polymorphisms and the effect on phenytoin dosage requirements."
ref. 17 Jose L et al. Acenocoumarol	1	A 29-year-old woman suffered vomiting, dizziness and neurological symptoms that were suggestive of phenytoin intoxication 3 weeks after starting phenytoin with a single	

and phenytoin toxicity in the presence of CYP2C9 mutation. J Assoc Physicians India 2008;56:250-2.	*3/*3: D	dose of 18 mg/kg, followed by 100 mg 3x daily. Co-medication: acenocoumarol 0.5 mg/day. The phenytoin plasma concentration was > 40 mg/L (reference values 10-20 mg/L). Gradual recovery occurred after withdrawal of phenytoin. The woman was found to be CYP2C9 *3/*3 and CYP2C19 IM.	
ref. 18 Ramasamy K et al. Severe phenytoin toxicity in a CYP2C9*3*3 homozygous mutant from India. Neurol India 2007;55:408-9.	2 *3/*3: D	A 22-year-old female had severe acute (nystagmus, ataxia and excessive sedation) and chronic (lymphadenopathy, pain and malformation of several bones, multiple bone fractures (suggestive of osteomalacia), anaemia, hirsutism, acne and gingival hypertrophy) symptoms of phenytoin intoxication 1 year after starting phenytoin 200-300 mg/day. The phenytoin plasma concentration was 33.2 mg/L at a dose of 300 mg/day. The woman was found to be *3/*3. The acute symptoms decreased immediately after withdrawal of phenytoin. The chronic symptoms were also significantly reduced after 6 months.	Authors' conclusion: "We recommend that wherever possible, the clinicians may do CYP2C9 genotyping of the epileptic patients before prescribing phenytoin."
ref. 19 Lee SY et al. Contributions of CYP2C9/CYP2C19 genotypes and drug interaction to the phenytoin treatment in the Korean epileptic patients in the clinical setting. J Biochem Mol Biol 2007;40:448-52.	3 *1/*3: A *3/*3: AA	97 patients, 1x *3/*3, 9x *1/*3, 87x *1/*1 (of which 36x CYP2C19 NM, 44x CYP2C19 IM and 7x CYP2C19 PM), phenytoin 3.6-6.1 mg/kg per day, co-medication was present in 56 patients. *1/*3 versus *1/*1 (both CYP2C19 NM or IM): - Decrease in the dose by 16% (S, from 5.58 to 4.67 mg/kg per day). - Increase in the trough concentration by 64% (S, from 1.79 to 2.92 mg/L). - Increase in the dose-corrected trough concentration by 95% (S, from 0.32 to 0.63 kg/L). *3/*3 versus *1/*1 (both CYP2C19 NM): - Decrease in the dose by 20% (NS, from 5.62 to 4.47 mg/kg per day). - Increase in the trough concentration by 263% (NS, from 1.45 to 5.27 mg/L). - Increase in the dose-corrected trough concentration by 357% (NS, from 0.26 to 1.18 kg/L). Multivariate regression analysis showed the CYP2C9 genotype to be an independent variable that contributed to the prediction of the phenytoin plasma concentration. The relationship between the CYP2C9 genotype and the phenytoin plasma concentration was present in patients with and without co-medication.	Authors' conclusion: "In a multiple regression model, the CYP2C9 and CYP2C19 genotypes and the phenytoin dosage were independent and statistically significant factors contributing to the total variability in serum phenytoin levels. However, they only accounted for 39.6% of the total variability in serum phenytoin levels." C_{ss}[b] versus *1/*1: *1/*3: 195% *3/*3: 457%
ref. 20 Rosemary J et al. Influence of the CYP2C9 and CYP2C19 polymorphisms on phenytoin hydroxylation in healthy individuals from south India. Indian J Med Res 2006;123:665-70.	3 *3/*3: AA *1/*3: AA *1/*2: AA	27 healthy volunteers, 1x *3/*3, 11x *1/*3, 5x *1/*2, 10x *1/*1, 300 mg single doses of phenytoin, no co-medication; Significant correlation between CYP2C9 genotype and phenytoin/p-HPPH metabolic ratio (r = 0.472, 95% CI 0.100-0.728). Significant correlation between combined CYP2C9+CYP2C19 genotype and phenytoin/p-HPPH metabolic ratio (r = 0.507, 95% CI 0.146-0.749). No difference in ratio between the different CYP2C19 genotypes. - *3/*3: increase in phenytoin/p-HPPH ratio versus *1/*1 from 34.8 to 109.9 (NS by 216%). - *1/*3: increase in phenytoin/p-HPPH ratio versus *1/*1 from 34.8 to 69.8 (NS by 101%). - *1/*2: increase in phenytoin/p-HPPH ratio versus *1/*1 from 34.8 to 68.2 (NS by 96%).	

ref. 21 Allabi AC et al. CYP2C9, CYP-2C19, ABCB1 (MDR1) genetic polymorphisms and phenytoin metabolism in a Black Beninese population. Pharmacogenet Genomics 2005;15:779-86.	3 AA	109 healthy volunteers, 53x *1/*1, 2x *1/*5, 3x *1/*6, 13x *1/*8, 21x *1/*9, 3x *1/*11, 1x *5/*6, 1x *5/*8, 2x *6/*9, 1x *8/*11, 4x *9/*8, 3x *9/*9, 2x *9/*11, 300 mg single doses of phenytoin, no co-medication; No significant differences in phenytoin plasma concentration between CYP2C9 genotypes. Significant association between CYP2C9 genotype and urinary excretion of (S)-p-HPPH. Significant difference in metabolic ratio of phenytoin (ratio (S)-p-HPPH in urine/plasma concentration of phenytoin) between CYP2C9 genotypes. Note: Significant association between ABCB1, MDR1 block-2 genotype and plasma concentration.	
ref. 22 Janssen P et al. De rol van CYP. Farmacogenetica en kinetiek van fenytoïne [The role of CYP. Pharmacogenetics and kinetics of phenytoin]. Pharm Weekblad 2005;37:1132-5.	3 *1/*2+ *1/*3: A	60 patients, 31x *1/*1 for both 2C9 and 2C19, 18x *1/variant for CYP2C9, ≥ 6-month fixed dose of phenytoin, co-medication known; The CYP2C9 *1/mutation genotype determined V_{max}. K_m was fixed as starting value based on *in vitro* data. Calculated dose recommendation for a plasma concentration of 14 mg/L based on a kinetic population model: 316 mg/day for CYP2C9 *1/*1, 220 mg/day for CYP2C9*1/mutation.	KNMP comment: The same study population as Van der Weide 2001. Authors' conclusion: "However, the distribution of the calculated concentrations for a genotype-specific dose is so great, that it remains essential to determine phenytoin concentrations in order to refine the phenytoin dose."
ref. 23 Tate SK et al. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. Proc Natl Acad Sci USA 2005;102:5507-12.	3 *3/*3: A *1/*2, *2/*2: AA	281 patients, 1x *3/*3, 39x (*1 or 2)/*3, 229x (*1 or *2)/(*1 or *2), phenytoin for ≥ 6 months, unknown co-medication; - *3: Significant association between *3 allele and max. required dose. Max. dose was 250 mg for *3/*3, 309 mg for 1x *3 and 354 mg for 0x *3. - *2: No significant association between *2 allele and max. dose. Note: genotyping was also performed for ABCB1 and SCN1A (gene that encodes the phenytoin target). Significant association between SCN1A and dose, no association between ABCB1 and dose.	
ref. 24 Hung CC et al. Dosage recommendation of phenytoin for patients with epilepsy with different CYP2C9/ CYP2C19 polymorphisms. Ther Drug Monit 2004;26:534-40.	3 *1/*3: A *1/*1: A	169 patients, 18x CYP2C9*1/*3 (1x CYP2C19 *2/*3, 2x *1*3, 9x *1/*2, 6x *1/*1), 151x CYP2C9 *1/*1 (6x CYP2C19 *2/*3, 10x *2/*2, 9x *1/*3, 79x *1/*2, 47x *1/*1), phenytoin for ≥ 1 month, other anti-epileptics as co-medication; - 2C9*1/*3 + 2C19*1/*1, *1/*2, *1/*3: decrease in dose[a] versus 2C9*1/*1 + 2C19*1/*1 from 5.3 to 4.1 mg/day/kg (S by 23%) and versus 2C9*1/*1 + 2C19*1/*2, *1/*3 from 4.9 to 4.1 (S by 16%). Increased C/D ratio versus 2C9*1/*1 + 2C19*1/*1 from 2.7 to 4.3 (S by 59%) and versus 2C9*1/*1 + 2C19*1/*2, *1/*3 from 3.0 to 4.3 (S by 43%). - 2C9*1/*1 + 2C19*2/*2, *2/*3: decrease in dose[a] versus 2C9*1/*1 + 2C19*1/*1 from 5.3 to 4.3 mg/day/kg (S by 19%) and versus 2C9*1/*1 + 2C19*1/*2, *1/*3 from 4.9 to 4.3 (NS by 12%). Increased C/D ratio versus 2C9*1/*1 + 2C19*1/*1 from 2.7 to 4.0 (S by 48%) and	Authors' conclusion: "The results revealed that the CY2C9 and CYP2C19 polymorphisms have dramatic effects on the population pharmacokinetic parameters of phenytoin, especially for CYP2C9." C_{ss} versus *1/*1: *1/*3: 125%.

ref. 24, continuation		versus 2C9*1/*1 + 2C19*1/*2, *1/*3 from 3.0 to 4.0 (NS by 33%). Note: anti-epileptics as co-medication do not appear to have a significant effect on dose and C/D ratio.	
ref. 25 Soga Y et al. CYP2C polymorphisms, phenytoin metabolism and gingival overgrowth in epileptic subjects. Life Sci 2004;74:827-34.	3 *1/*3: AA	28 patients, phenytoin for > 5 years, 3x *1/*3, 25x *1/*1, and 56 healthy volunteers, 3x *1/*3, 53x *1/*1, co-medication unknown; *clinical endpoints* No significant relationship between CYP2C9 or CYP2C19 genotype and severity of the side effect gingival hyperplasia. *kinetic endpoints* - CYP2C9*1/*3: increased C/D ratio versus *1/*1 (S).	Authors' conclusion: "Based on the results, we concluded that CYP2C9*3 polymorphism influenced phenytoin metabolism and subsequent C/D ratio, while CYP2C19 polymorphisms had minor effect."
ref. 26 Kidd RS et al. Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. Pharmacogenetics 2001;11:803-8.	2 *6/*6: D	Single patient, *6*/6, symptoms of phenytoin intoxication (confusion, slurred speech, memory loss, inability to stand) 13 days after initiation of phenytoin 100 mg 3x daily. No relevant co-medication, CYP2C19 genotype was *1/*1. Kinetic parameters: plasma concentration on day 1 of intoxication was 49.5 mg/L, t½ was 13 hours, AUC was 895 mg·day/mL, 5.8x higher than theoretical NM, Cl_{or} was approx. 17% of a theoretical NM.	
ref. 27 Brandolese R et al. Severe phenytoin intoxication in a subject homozygous for CYP2C9 *3. Clin Pharmacol Ther 2001;70:391-4.	2 *3/*3: D	Single patient, *3*/3, symptoms of phenytoin intoxication (dysarthria, dysmetria, dyskinesia, nystagmus, altered mental status) 10 days after initiation of phenytoin 100 mg 3x daily. No relevant co-medication, CYP2C19 genotype was *1/*2. Kinetic parameters: plasma concentration on day 1 of intoxication was > 100 mg/L, t½ was 103 hours (5x higher than for NM). Complete recovery after withdrawal of phenytoin.	
ref. 28 Caraco Y et al. Phenytoin metabolic ratio: a putative marker of CYP2C9 activity in vivo. Pharmacogenetics 2001;11:587-96.	3 *2/*3: AA *2/*2: AA *1/*3: A *1/*2: A	A total of 31 healthy volunteers, 1x *2/*3, 1x *2/*2, 4x *1/*3, 7x *1/*2, 18x *1/*1, 300 mg single doses of phenytoin, no co-medication; - *2/*3: increase in the AUC versus *1/*1 from 472 to 1260 µM/h (NS by 167%), decrease in Cl_{or} from 28.9 to 3.3 mL/min (NS by 89%), increase in t½ from 17.0 to 63.0 hours (NS by 271%). - *2/*2: increase in the AUC versus *1/*1 from 472 to 1271 µM/h (NS by 169%), decrease in Cl_{or} from 28.9 to 4.8 mL/min (NS by 83%), increase in t½ from 17.0 to 28.5 hours (NS by 77%). - *1/*3: increase in the AUC versus *1/*1 from 472 to 697 µM/h (S by 48%), decrease in Cl_{or} from 28.9 to 12.5 mL/min (S by 57%), increase in t½ from 17.0 to 26.4 hours (S by 55%). - *1/*2: increase in the AUC versus *1/*1 from 472 to 705 µM/h (S by 49%), decrease in Cl_{or} from 28.9 to 13.9 mL/min (S by 52%), increase in t½ from 17.0 to 21.2 hours (S by 25%).	AUC versus *1/*1: *1/*2: 149% *1/*3: 148% *2/*2: 269% *2/*3: 267%
ref. 29 Van der Weide J et al. The effect of genetic polymorphism of cytochrome	4 *1/*2, *2/*2, *2/*3: A	60 patients, 3x *2/*2, 2x *2/*3, 9x *1/*3, 9x *1/*2, 37x *1/*1, ≥ 6-month fixed dose of phenytoin, co-medication known; - 1 or 2 mutant alleles: decrease in daily dose versus *1/*1 from 287 to 199 mg/day (S by 31%). For patients with a serum concentration between 10 and 20 mg/L,	

P450 CYP2C9 on phenytoin dose requirement. Pharmacogenetics 2001;11:287-91. *Results were also published in: Pharm Weekblad 2005;16:565-9 and in Ned Tijdschr Geneeskd 2001;145:312-5.*		decrease in mean dose from 314 to 199 mg/day (S by 37%). Note: no significant difference in daily dose between the groups with and without co-medication, both for patients with and without mutant alleles. No significant difference in dose between carriers and non-carriers of the CYP2C19*2 allele.	
ref. 30 Kerb R et al. The predictive value of MDR1, CYP2C9, and CYP2C19 polymorphisms for phenytoin plasma levels. Pharmacogenomics J 2001;1:204-10.	3 *2/*2, *3/*3: A *1/*2, *1/*3: A	96 healthy volunteers, CYP2C9 genotypes: 1x *3/*3, 3x *2/*2, 15x *1/*3, 13x *1/*2, 64x *1/*1, CYP2C19 genotypes: 3x *2/*2, 16x *1/*2, 75x *1/*1, 300 mg single doses of phenytoin, no co-medication; - CYP2C9*2/*2+*3/*3: p-HPPH/phenytoin ratio decreased from 1.83 to 0.76 versus *1/*1 (S by 58%). Increased phenytoin concentration versus *1/*1, from 4.20 to 6.41 mg/L (S by 53%). - CYP2C9*1/*2+*1/*3: p-HPPH/phenytoin ratio decreased from 1.83 to 1.34 versus *1/*1 (S by 27%). Increased phenytoin concentration versus *1/*1, from 4.20 to 5.53 mg/L (S by 32%). Number of mutant CYP2C9 alleles accounts for 14% of the variation in phenytoin plasma concentration. No significant association between CYP2C19*2 allele and concentration of phenytoin or p-HPPH/phenytoin ratio. A total of 35 patients from TDM programme, using phenytoin for > 1 month, co-medication unknown; C/D ratio increases with number of mutant CYP2C9 alleles.	Authors' conclusion: "A combined analysis of variable alleles of CYP2C9, 2C19 and MDR1 revealed that the number of mutant CYP2C9 alleles is a major determinant, the number of MDR 18T alleles further contributes to the prediction of phenytoin plasma levels and CYP2C19*2 does not explain individual variability."
ref. 31 Ninomiya H et al. Genetic polymorphism of the CYP-2C subfamily and excessive serum phenytoin concentration with central nervous system intoxication. Ther Drug Monit 2000;22:230-2.	2 *1/*3: D	Single patient, CYP2C9*1*/3, symptoms of phenytoin intoxication (symptoms of CNS intoxication, ataxia, diplopia) with use of phenytoin 187.5 mg/day, plasma concentration was 32.6 mg/L. No relevant co-medication, CYP-2C19 genotype was *1/*3.	
ref. 32 Aynacioglu AS et al. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Br J Clin Pharmacol 1999;48:409-15.	3 *3/*3: AA *1/*3: A *2/*2: A	101 healthy volunteers, 1x *3/*3, 16x *1/*3, 3x *2/*2, 13x *1/*2, 68x *1/*1, 300 mg single doses of phenytoin, no co-medication; - *3/*3: increased phenytoin concentration versus *1/*1, from 4.16 to 5.92 mg/L by 42%, decrease in p-HPPH/phenytoin ratio from 0.43 to 0.02 by 95%. Significances unknown. - *1/*3: increased phenytoin concentration versus *1/*1, from 4.16 to 5.65 mg/L (S by 36%), decrease in p-HPPH/phenytoin ratio from 0.43 to 0.21 (S by 51%). - *2/*2: increased phenytoin concentration versus *1/*1, from 4.16 to 6.58 mg/L (S by 58%), decrease in p-HPPH/phenytoin ratio from 0.43 to 0.14 (S by 67%). - *1/*2: increased phenytoin concentration versus *1/*1,	Phenytoin concentration versus *1/*1: *1/*2: 133% *1/*3: 136% *2/*2: 158% *3/*3: 142%

ref. 32, continuation	*1/*2: A	from 4.16 to 5.52 mg/L (S by 33%), decrease in p-HPPH/phenytoin ratio from 0.43 to 0.26 (S by 40%).	
ref. 33 Kidd RS et al. Pharmacokinetics of chlorpheniramine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. Pharmacogenetics 1999;9:71-80.	2 *3/*3: A	24 healthy volunteers, 1x CYP2C9*3/*3 CYP2C19 *1/*1, 23x unknown genotype, 100 mg single doses of phenytoin, no co-medication; - *3/*3: increase in Cmax versus unknown genotype from 1.72 to 2.33 µg/L (S by 35%), increase in t½ from 14.1 to 46.5 hours (S by 230%), decrease in Cl_{or} from 2.35 to 0.50 L/hour (S by 79%), increase in AUC from 46.9 to 199.6 µg·h/L (S by 326%).	AUC versus *1/*1 (unknown genotype): *3/*3: 426%
ref. 34 Mamiya K et al. The effects of genetic polymorphisms of CYP2C9 and CYP2C19 on phenytoin metabolism in Japanese adult patients with epilepsy: studies in stereoselective hydroxylation and population pharmacokinetics. Epilepsia 1998;39:1317-23.	3 *1/*3: AA	134 patients, 3x CYP2C9*1/*3+ CYP2C19*1/*1, 15x CYP2C9*1/*1+CYP2C19*2/*2, *3/*3 or *2/*3, 64x CYP2C9 *1/*1+CYP2C19 *1/*2 or *1/*3, 52x CYP2C9*1/*1+CYP2C19 *1/*1, ≥ fixed dose of phenytoin for 1 month, with co-medication; - 2C9*1/*3+2C19*1/*1: decrease in dose[a] versus 2C9*1/*1+2C19*1/*1 from 3.58 to 2.09 mg/day/kg (NS by 42%), increase in C/D ratio from 1.7 to 2.2 (NS by 29%). - 2C9*1/*1+2C19*2/*2, *3/*3 or *2/*3: decrease in dose[a] versus 2C9*1/*1+2C19*1/*1 from 3.58 to 2.95 mg/day/kg (NS by 18%), increase in C/D ratio from 1.7 to 2.2 (NS by 29%). - 2C9*1/*1+2C19*1/*2, *1/*3: decrease in dose[a] versus 2C9*1/*1+2C19*1/*1 from 3.58 to 3.50 mg/day/kg (NS by 2%), increase in C/D ratio from 1.7 to 2.3 (NS by 35%).	C_{ss} versus *1/*1: *1/*3: 71%
ref. 35 Odani A et al. Genetic polymorphism of the CYP2C subfamily and its effect on the pharmacokinetics of phenytoin in Japanese patients with epilepsy. Clin Pharmacol Ther 1997;62:287-92.	3 *1/*3: A	44 patients, 1-33 years of age, 6x CYP2C9*1/*3, 38x CYP2C9*1/*1, mean phenytoin dose 5.18 mg/kg/day, co-medication known; - CYP2C9*1/*3: V_{max} was 33% lower than in CYP2C9 *1/*1. - CYP2C19*1/*2, *1/*3, *2/*2, *2/*3: V_{max} was 14% lower than for CYP2C19*1/*1. - Note: co-medication with carbamazepine and phenobarbital has no effect on pharmacokinetic parameters of phenytoin.	
ref. 36 Hashimoto Y et al. Effect of CYP2C polymorphisms on the pharmacokinetics of phenytoin in Japanese patients with epilepsy. Biol Pharm Bull 1996;19:1103-5.	3 *1/*3: AA	17 patients, mean 5.30 mg/kg/day phenytoin, anti-epileptics as co-medication; - 2C9*1/*3+2C19 *1/*1: decrease in V_{max} versus 2C9*1/*1+2C19*1/*1 from 10.4 to 6.2 mg/kg/day (by 40%). No change in K_m and volume of distribution. - 2C9*1/*1+2C19*2/*2 or *2/*3: decrease in V_{max} versus 2C9*1/*1+2C19*1/*1 from 10.4 to 8.9 mg/kg/day (by 14%). No change in K_m and volume of distribution.	
ref. 37 SmPC Diphantoïne-Z (phenytoin) 01-11-21.	0	Warning: *Anticonvulsant Hypersensitivity Syndrome, AHS* Life-threatening cutaneous reactions, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), have been reported during phenytoin use. Patient-controlled, genome-wide association-studies in Taiwanese, Japanese, Malay and Thai patients showed an increased risk of severe cutaneous adverse reactions	

ref. 37, continuation	*1/*3+ *3/*3: E	(SCARs) in carriers of the CYP2C9*3 variant with decreased functionality. *CYP2C9 metabolism* Phenytoin is metabolised by the CYP450 enzyme CYP2C9. Patients who are carrier of the CYP2C9*2 or CYP2C9*3 variant with decreased functionality (intermediate or poor metabolisers of CYP2C9 substrates) can have a higher risk of increased phenytoin plasma concentrations and resulting toxicity. For patients know to be carrier of the CYP2C9*2 or CYP2C9*3 allele with decreased functionality, careful monitoring of the clinical response is recommended. It might be necessary to monitor the phenytoin plasma concentrations. <u>Adverse events</u>: Severe cutaneous reactions Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are reported very rarely. In case of occurrence of these reactions, it is necessary to discontinue phenytoin treatment. Toxicodermia with eosinophilia and systemic symptoms (DRESS): individual reports indicate that, although they remain very rare, the number of hypersensitivity reactions, like skin rash and liver toxicity, is increased in Blacks.	
ref. 38 SmPC Dilantin (phenytoin), USA, 03-03-22.	0 *1/*3+ *3/*3: E	<u>Warning</u>: Serious dermatologic reactions Dilantin can cause severe cutaneous adverse reactions (SCARs), which may be fatal. Retrospective, case-control, genome-wide association studies in patients of southeast Asian ancestry have also identified an increased risk of SCARs in carriers of the decreased function CYP2C9*3 variant, which has also been associated with decreased clearance of phenytoin. Consider avoiding DILANTIN as an alternative to carbamazepine in CYP2C9*3 carriers. The use of CYP2C9 genotyping has important limitations and must never substitute for appropriate clinical vigilance and patient management. <u>Use in specific populations</u>: Use in patients with decreased CYP2C9 function Patients who are intermediate or poor metabolizers of CYP2C9 substrates (e.g., *1/*3, *2/*2, *3/*3) may exhibit increased phenytoin serum concentrations compared to patients who are normal metabolizers (e.g., *1/*1). Thus, patients who are known to be intermediate or poor metabolizers may ultimately require lower doses of phenytoin to maintain similar steady-state concentrations compared to normal metabolizers. If early signs of dose-related central nervous system (CNS) toxicity develop, serum concentrations should be checked immediately <u>Clinical pharmacology</u>: *Pharmacokinetics* Phenytoin is primarily metabolized by the hepatic cytochrome P450 enzyme CYP2C9 and to a lesser extent by CYP2C19. Because phenytoin is hydroxylated in the liver by an enzyme system which is saturable at high serum levels, small incremental doses may increase the half-life and produce very substantial increases in serum levels, when these are in the upper range. The steady-state level may be disproportionately increased, with resultant intoxication, from an increase in dosage of 10% or more. Unusually high levels result from liver disease, variant CYP2C9 and CYP2C19 alleles, or drug interactions which result in metabolic interference. *Pharmacogenomics* CYP2C9 activity is decreased in individuals with genetic variants such as the CYP2C9*2 and CYP2C9*3 alleles.	

ref. 38, continuation		Carriers of variant alleles, resulting in intermediate (e.g., *1/*3, *2/*2) or poor metabolism (e.g., *2/*3, *3/*3) have decreased clearance of phenytoin. Other decreased or nonfunctional CYP2C9 alleles may also result in decreased clearance of phenytoin (e.g., *5, *6, *8, *11). The prevalence of the CYP2C9 poor metabolizer phenotype is approximately 2-3% in the White population, 0.5-4% in the Asian population, and <1% in the African American population. The CYP2C9 intermediate phenotype prevalence is approximately 35% in the White population, 24% in the African American population, and 15-36% in the Asian population.	

[a] Corrected for body weight.
[b] Corrected for dose

Risk group	*1/*2, *1/*3, *2/*2, *2/*3 and IM with CYP2C9 inhibitors

Comments:

- The only clinical studies included from 2009 are those that included more than 100 patients. Studies investigating cutaneous adverse events were only included if data were (also) provided for severe cutaneous adverse events. Cutaneous adverse events cannot be prevented by dose reduction and the reversibility of mild cutaneous adverse events and low predictive value of CYP2C9 variants for cutaneous adverse events do not warrant avoiding phenytoin in patients with an increased risk. As a result, a therapeutic recommendation is not possible based on data on mild cutaneous adverse events. Kinetic studies were only included if the (dose-corrected) AUC, plasma concentration or clearance was determined per genotype and there were at least three *1/*2 or *1/*3 or at least one *2/*2, *2/*3 or *3/*3. Other articles do not contribute sufficiently to the burden of proof.
- Taguchi M et al. (Drug Metab Pharmacokinet 2005;20:107-12) found no significant effect of the CYP2C9 genotype on V_{max} or on V_{max}/K_m. The study population consisted of 20 patients, both children and adults (2-25 years), 18x *1/*1 and 2x *1/*3.
- Existing guidelines:
  Caudle KE et al. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. Clin Pharmacol Ther 2014;96:542-8. PubMed PMID: 25099164 and Karnes JH et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C9 and HLA-B Genotypes and Phenytoin Dosing: 2020 Update. Clin Pharmacol Ther 2021;109:302-9. PMID: 32779747.
  CPIC indicates that *in vitro* and clinical studies suggest that CYP2C9 decreased function and no or almost no function alleles generate variant enzymes with activities that are substrate-dependent. Therefore, assigning function to CYP2C9 alleles requires careful evaluation of individual drugs. Despite this, CPIC does not distinguish between the *1/*3 and *2/*2 genotypes and between the *2/*3 and *3/*3 genotypes. CPIC assigns an activity score of 1 to fully functional CYP2C9 alleles, an activity score of 0.5 to CYP2C9 alleles with reduced but not (almost) no function like *2, and an activity score of 0 to CYP2C9 alleles with (almost) no function like *3. CPIC groups genotypes into phenotypes based on activity score and sometimes distinguishes different activity scores within a phenotype. The CPIC phenotype normal metaboliser (NM) only contains gene activity score 2 (*1/*1). The CPIC phenotype intermediate metaboliser (IM) contains gene activity scores 1 (including both *1/*3 and *2/*2) and 1.5 (including *1/*2), so CPIC never distinguishes between *1/*3 and *2/*2. CPIC indicates that allocation of *1/*3 and *2/*2 into one group is based on data for multiple substrates (flurbiprofen, celecoxib, phenytoin, and warfarin) showing a similar effect of *1/*3 and *2/*2 on metabolic ratio and dose requirements (warfarin) (Vogl S et al. CYP2C9 genotype vs. metabolic phenotype for individual drug dosing - a correlation analysis using flurbiprofen as probe drug. PLoS One 2015;10:e0120403; Kusama M et al. Prediction of the effects of genetic polymorphism on the pharmacokinetics of CYP2C9 substrates from in vitro data. Pharm Res 2009;26: 822-35 PubMed: 19082874; and Lindh JD et al. Influence of CYP2C9 genotype on warfarin dose requirements - a systematic review and meta-analysis. Eur J Clin Pharmacol 2009;65:365-75. PubMed: 19031075). The CPIC phenotype poor metaboliser (PM) contains gene activity scores 0 (including *3/*3) and 0.5 (including *2/*3). So, CPIC can distinguish between *2/*3 and *3/*3, but does not do so for phenytoin. The summary below follows the KNMP definitions for IM (i.e. IM OTHER) and PM (i.e. PM OTHER).
  CPIC indicates that there is substantial evidence linking CYP2C9 genotypes with phenotypic variability. CPIC states that the available literature provides consistent, high quality evidence for the relationship between genetic variation and phenotypic variability. CPIC originally mentioned that available estimates from models indicate that CYP2C9 variant alleles lead to lower phenytoin clearance, depending on the allele and the number of alleles. Several studies have shown that *1/*2 and *1/*3 lead to moderate decreases in clearance. Phenytoin maintenance doses were decreased by 23-38% versus *1/*1 in these genotypes (Hung 2004, Van der Weide 2001, Hung 2012). In the update, references were only included in the supplementary files. CPIC indicates that availa-

ble evidence does not clearly indicate the extent of dose reduction needed to prevent phenytoin-related toxicities in *1/*3, *2/*2, IM OTHER with an (almost) no function allele, and PM OTHER without an (almost) no function allele. CPIC indicates that multiple case studies have observed an increased risk for exposure-related phenytoin toxicities (originally mentioned references: Brandolese 2001, Ramasamy 2007, Hennessy 2009 and Dorado 2013; last reference not included in our risk analysis), and that multiple studies have observed an association between the *3 allele and SJS/TEN (Yampayon 2017, Tassaneeyakul 2016, and Chung 2014). Although carriage of the CYP2C9*3 allele is insufficient to predict phenytoin-induced SJS/TEN, these and other data suggest that the risk of SJS/TEN is dose-related and provide an additional rationale for reducing phenytoin dose in patients with two reduced function alleles of which at least one an (almost) no function allele (Karnes JH et al. Applications of immunopharmacogenomics: predicting, preventing, and understanding immune-mediated adverse drug reactions. Annu Rev Pharmacol Toxicol 2019;59:463-86. PubMed: 30134124). CPIC indicates that available evidence does not clearly indicate the extent of dose reduction needed to prevent phenytoin-related toxicities in these patients.

CPIC indicates that (fos)phenytoin dose should first be adjusted according to a patient's clinical characteristics. In patients with *1/*2 or in IM OTHER with the reduced function allele not being an (almost) no function allele, CPIC indicates that the recommended phenytoin initial or loading and maintenance doses do not need adjustments based on genotype. CPIC classifies this recommendation as moderate, meaning that there is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects. CPIC indicates that their dose recommendations are conservative given the variability surrounding phenytoin dosing. Based on the doses reported in the pharmacokinetic and pharmacogenetic studies (Hung 2012, Hung 2004, Van der Weide 2001, and other studies mentioned only in the supplementary files), CPIC states that a typical initial or loading dose followed by at least a 25% reduction in the recommended starting maintenance dose may be considered for *1/*3, *2/*2, IM OTHER with an (almost) no function allele, and PM OTHER without an (almost) no function allele. Subsequent maintenance doses should be adjusted based on therapeutic drug monitoring and response. CPIC classifies this recommendation as moderate, meaning that there is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects. For *2/*3, *3/*3 and PM OTHER with at least one (almost) no function allele, CPIC recommends to use a typical initial or loading dose then to consider at least a 50% reduction of starting maintenance dose with subsequent maintenance doses adjusted based on therapeutic drug monitoring and response. CPIC classifies this recommendation as strong, meaning that the evidence is high quality and the desirable effects clearly outweigh the undesirable effects.

CPIC indicates that, while limited data are available for effects of CYP2C9 alleles on phenytoin metabolism in paediatric patient populations, there is no compelling data to indicate that CYP2C9 polymorphisms will affect phenytoin metabolism differently in children compared to adults. As such, the paediatric recommendation was extrapolated using adult data.

CPIC indicates that some studies suggest that variants in other genes also contribute to altered phenytoin metabolism (e.g., CYP2C19, CYP1A1, and EPHX1 (reviewed in Thorn CF et al. PharmGKB summary: phenytoin pathway. Pharmacogenet Genomics 2012;22:466-70. PubMed: 22569204)) and combined genetic analysis might improve the prediction of phenytoin metabolism (Hung 2012 and Hung 2004). However, these studies evaluating the effect of multiple gene variation and phenytoin dose requirements are limited and have not been replicated. Consequently, the CPIC guideline on genotype-directed phenytoin dosing is limited to CYP2C9.

CPIC indicates that if both HLA-B*1502 and CYP2C9 genotypes are known, the HLA-B*1502 genotype should be considered first, then CYP2C9 genotype.

The recommendations for patients with variant CYP2C9 genotypes are as follows:

CYP2C9 genotype	Therapeutic recommendation	Classification of recommendation
*1/*2 and IM OTHER with the reduced function allele not being an (almost) no function allele	No adjustments needed from typical dosing strategies. Subsequent doses should be adjusted according to therapeutic drug monitoring, response and side effects. An HLA-B*1502 negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and patients should be carefully monitored according to standard practice. (i.e. the same recommendation as given for *1/*1.)	Moderate[a]
*1/*3, *2/*2, and IM OTHER with an (almost) no function allele, and PM OTHER without an (almost) no function allele	For first dose, use typical initial or loading dose. For subsequent doses, use approximately 25% less than typical maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring, response and side effects. An HLA-B*1502 negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and patients should be carefully monitored according to standard practice.	Moderate[a]

*2/*3, *3/*3, and PM OTHER with at least one (almost) no function allele	For first dose, use typical initial or loading dose. For subsequent doses, use approximately 50% less than typical maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring, response and side effects. An HLA-B*1502 negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and patients should be carefully monitored according to standard practice.	Strong[b]

[a]: There is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.
[b]: The evidence is high quality and the desirable effects clearly outweigh the undesirable effects.
On 3-5-2022, there was not a more recent version of the recommendations present on the CPIC-site.

Date of literature search: 23 April 2022.

	Genotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	*1/*2	4 D	yes	yes	23 May 2022
	*1/*3	4 F	yes	yes	
	*2/*2	4 D	yes	yes	
	*2/*3	4 D	yes	yes	
	*3/*3	4 F	yes	yes	
	IM	-	yes	yes	
	PM	2 D	yes	yes	

Mechanism:
Phenytoin is predominantly metabolised by CYP2C9 (90%) and also by CYP2C19 (10%), to the inactive metabolite para-hydroxyphenytoin (5-(para-hydroxyphenyl)-5-phenylhydantoin, p-HPPH). Formation of this metabolite goes through an unstable arene-oxide intermediate.
Unlike most side effects are the immune-mediated cutaneous adverse events usually (largely) concentration independent. Analogy with other drug-induced cutaneous adverse events suggests the following mechanism. A cellular immune reaction against tissue cells is induced if peptides derived from proteins within these tissue cells bind to specific HLA proteins, are transported to the cell surface and are ”recognised” as foreign by specific immune cell proteins (T-cell receptors). (A metabolite of) phenytoin binds to either the cellular proteins or derived peptides, to specific HLA proteins or to specific T-cell receptors, thus inducing an interaction between an HLA peptide complex and a T-cell receptor, resulting in a cellular immune reaction against tissue cells. Thus, the effect of CYP2C9 variants on the risk of severe cutaneous adverse events is most likely not mediated by the increased phenytoin concentrations, but by a change in metabolites formed or a disbalance in the rates of formation and detoxification of the unstable arene-oxide intermediate.
Phenytoin exhibits non-linear pharmacokinetics. With chronic use, phenytoin induces CYP450 enzymes, primarily CYP2C9 and CYP2C19. So, phenytoin induces its own metabolism. However, because CYP2C9 and CYP2C19 are saturable at high phenytoin serum concentrations, small incremental doses may produce very substantial increases in serum concentrations, when these are in the upper range.
The NVZA mentions the following therapeutic ranges: 8-20 µg/ml (total phenytoin) and 0.5-2 µg/ml (free phenytoin).
The NVZA mentions the following toxic concentrations: > 20 µg/ml (total phenytoin) and > 2 µg/ml (free phenytoin).
The NVZA indicates that plasma concentrations should be measured after at least 4-5 days.

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 consider genotyping 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+	8+
Corresponding Clinical Implication Score:		Essential