Review and Emerging Evidence on Transporter Polymorphisms

Kate Hillgren, Sr. Research Advisor
Lilly Research Labs
(i) genome-wide association studies have identified them to be significantly associated with the pharmacokinetics or pharmacodynamics of one or more drugs at genome-wide level significance

(ii) multiple candidate gene studies have identified significant associations between these polymorphisms and drug disposition, efficacy, or toxicity

(iii) the polymorphisms have exhibited functional changes in in vitro studies

♦ SLC01B1 encoding OATP1B1
  • c.521T>C, p.V174A, rs4149056

♦ BCRP (ABCG2)
  • c.421C>A, p.Q141K, rs2231142

Importance of OATP1B1

Drugs Interacting with OATP1B1
- Statins (S,I)
- Repaglinide (S, I)
- Atrasentan (S)
- Rosiglitazone (I)
- Fexofenadine (S)
- Bile Acids (S,I)
- Rifampin (S, I)
- Cyclosporin A (I)
- Gemfibrozil (I)

S- Substrate
I- Inhibitor
## SLC01B1 Variants Leading to Amino Acid Changes in OATP1B1


<table>
<thead>
<tr>
<th>SLC01B1 Allele</th>
<th>Polymorphisms</th>
<th>Protein Variation</th>
<th>Consequence</th>
<th>NA</th>
<th>EA</th>
<th>S/CA</th>
<th>ME</th>
<th>EU</th>
<th>AM</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>1a</em></td>
<td>*1 Reference Allele, wt</td>
<td>NA</td>
<td>NA</td>
<td>34</td>
<td>25</td>
<td>52</td>
<td>49</td>
<td>56</td>
<td>37</td>
<td>21</td>
</tr>
<tr>
<td><em>1b</em></td>
<td>388A&gt;G</td>
<td>Asn130Asp</td>
<td>Increased activity?</td>
<td>48</td>
<td>63</td>
<td>39</td>
<td>31</td>
<td>26</td>
<td>39</td>
<td>77</td>
</tr>
<tr>
<td><em>5</em></td>
<td>521T&gt;C</td>
<td>Val174Ala</td>
<td>Decreased Function</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>15</em></td>
<td>388A&gt;G 521T&gt;C</td>
<td>Asn130Asp Val174Ala</td>
<td>Decrease Function</td>
<td>16</td>
<td>12</td>
<td>9</td>
<td>15</td>
<td>16</td>
<td>24</td>
<td>2</td>
</tr>
</tbody>
</table>

NA – North Africa, EA – East Asia, S/CA – South or Central Asia, ME – Middle East, EU – Europe, AM – America, SA – Sub-Saharan Africa
OATP1B1 521T>C Pharmacokinetic Impact

Individuals with Polymorphisms of OATP1B1 Have Higher Plasma Levels of Simvastatin Acid

Drugs Effected by OATP1B1 Genetic Variants
- Pravastatin
- Repaglinide
- Methotrexate
- Maraviroc
- Nateglinide
- Pitavastatin
- Simvastatin
- Rosuvastatin
- Lopinavir
- Erythromycin
- Flavopiridol
- Enalapril
- Atorvastatin

OATP1B1 Variants and Statin-induced Myopathy

- Genome Wide Association Study (GWAS) using approximately 300,000 markers
- 12,000 participants; all were taking simvastatin (80 mg) daily
- 85 subjects with definite or incipient myopathy and 90 controls
- No associations between myopathy and SNPs in any other region yielded an uncorrected P value of $< 10^{-5}$

Cumulative Risk of Myopathy Associated with SLCO1B1 Minor C Allele

521CC homozygotes:
- 18% cumulative risk
- Myopathy occurred primarily during the first year

521CT heterozygotes:
- 3% cumulative risk

521TT homozygotes:
- 0.6% cumulative risk

Overall, more than 60% of myopathy cases could be attributed to the 521T>C variant in OATP1B1.

Subjects on 80 mg simvastatin

Evidence from PharmGKB

Clinical Variants that meet the highest level of criteria, manually curated by PharmGKB, are shown below. Please follow the link in the "Position" column for more information about a particular variant. Each link in the "Position" column leads to the corresponding PharmGKB Variant Page. The Variant Page contains summary data, including PharmGKB manually curated information about variant-drug pairs based on individual PubMed publications. The PMIDs for these PubMed publications can be found on the Variant Page.

To see more Clinical Variants with lower levels of criteria, click the button at the bottom of the table.

**rs4149056 related to simvastatin - toxicity/adr (1A)**

<table>
<thead>
<tr>
<th>Level of Evidence</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1A</td>
<td>Patients with the CC genotype may have a higher risk of simvastatin-related myopathy as compared to patients with the CT or TT genotype. Other genetic and clinical factors may also influence a patient’s risk for toxicity.</td>
<td>Patients with the CT genotype may have a higher risk of simvastatin-related myopathy as compared to patients with the TT genotype. Other genetic and clinical factors may also influence a patient’s risk for toxicity.</td>
<td>Patients with the TT genotype may have a lower risk of simvastatin-related myopathy as compared to patients with the CT or CC genotype. Other genetic and clinical factors may also influence a patient’s risk for toxicity.</td>
</tr>
</tbody>
</table>

**Type**
- Toxicity/ADR

**Genes**
- SLC01B1

**Phenotypes**
- Muscular Diseases
- Myopathy
- Central Core

**OMB Race**
- Mixed Population

**Clinical Annotation Levels of Evidence**

- Level 1a: high
- Level 1b: moderate
- Level 2a: moderate
- Level 2b: low
- Level 3: low
- Level 4: preliminary
from search: **OATP1B1**

**GENE:**

**SLCO1B1**

solute carrier organic anion transporter family, member 1B1

### Dosing Guidelines (1) Drug Labels (0) Clinical Annotations (43)

#### 1. CPIC Guideline for simvastatin and SLCO1B1

**Table 1: Recommended dosing of simvastatin based on SLCO1B1 phenotype**

Adapted from Table 1 and 2 of the 2014 guideline update manuscript.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Examples of diplootypes a</th>
<th>Genotype at rs4149056</th>
<th>Implications for simvastatin</th>
<th>Dosing recommendations for simvastatin b,c</th>
<th>Classification of recommendations d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal function, Homozygous wild-type (two normal function alleles)</td>
<td>*1a/*1a, *1a/*1b, *1b/*1b</td>
<td>TT</td>
<td>Normal myopathy risk</td>
<td>Prescribe desired starting dose and adjust doses of simvastatin based on disease-specific guidelines.</td>
<td>Strong</td>
</tr>
<tr>
<td>Intermediate function, Heterozygous (one normal function allele plus one decreased function allele)</td>
<td>*1a/*5, *1a/*15, *1a/*17, *1b/*5, *1b/*15, *1b/*17</td>
<td>TC</td>
<td>Intermediate myopathy risk</td>
<td>Prescribe a lower dose or consider an alternative statin (e.g. pravastatin or rosuvastatin); consider routine CK surveillance.</td>
<td>Strong</td>
</tr>
<tr>
<td>Low function, Homozygous variant or mutant (two decreased function alleles)</td>
<td>*5/*5, *5/*15, *5/*17, *15/*15, *15/*17, *17/*17</td>
<td>CC</td>
<td>High myopathy risk</td>
<td>Prescribe a lower dose or consider an alternative statin (e.g. pravastatin or rosuvastatin); consider routine CK surveillance.</td>
<td>Strong</td>
</tr>
</tbody>
</table>

CK, creatine kinase.

a SLCO1B1 alleles are often named using * allele nomenclature, representing various SNPs alone or in combination ([http://www.pharmgkb.org/gene/PA1348665839#tabview=tab4&subtab=33](http://www.pharmgkb.org/gene/PA1348665839#tabview=tab4&subtab=33)) (2014 Update Supplemental Table S1) that are associated with low SLCO1B1 protein expression or function (2014 Update Supplemental Table S2). The minor C allele at rs4149056 is contained within SLCO1B1*5 (rs4149056 alone) as well as the *15 and *17 haplotypes and is associated with lower plasma clearance of simvastatin. The magnitude of this effect is similar for *5, *15, and *17 haplotypes.
GWAS Study on Low-Density Lipoprotein Cholesterol Response After Statin Treatment

Table 1 | Genome-wide significant associations in stage 1, stage 2 and combined meta-analysis.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Position</th>
<th>Lead SNP</th>
<th>Gene</th>
<th>Coding allele</th>
<th>Noncoding allele</th>
<th>Phase</th>
<th>N</th>
<th>Frequency-coding allele</th>
<th>Beta*</th>
<th>s.e.</th>
<th>% Extra reduction†</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109620053</td>
<td>rs646776</td>
<td>SORT1/ CELSR2/ PSRC1</td>
<td>C</td>
<td>T</td>
<td>Stage 1</td>
<td>16,697</td>
<td>0.230</td>
<td>−0.015</td>
<td>0.003</td>
<td>1.5</td>
<td>6.70 × 10^{-7}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stage 2</td>
<td>21,902</td>
<td>0.216</td>
<td>−0.010</td>
<td>0.003</td>
<td>1.0</td>
<td>2.43 × 10^{-4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td>38,599</td>
<td>0.218</td>
<td>−0.018</td>
<td>0.002</td>
<td>1.3</td>
<td>1.05 × 10^{-9}</td>
</tr>
<tr>
<td>6</td>
<td>160930108</td>
<td>rs10455872</td>
<td>LPA</td>
<td>G</td>
<td>A</td>
<td>Stage 1</td>
<td>12,981</td>
<td>0.069</td>
<td>0.041</td>
<td>0.006</td>
<td>−4.1</td>
<td>1.95 × 10^{-11}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stage 2</td>
<td>18,075</td>
<td>0.087</td>
<td>0.059</td>
<td>0.005</td>
<td>−5.9</td>
<td>7.41 × 10^{-35}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td>36,056</td>
<td>0.085</td>
<td>0.052</td>
<td>0.004</td>
<td>−3.2</td>
<td>7.41 × 10^{-44}</td>
</tr>
<tr>
<td>12</td>
<td>21260064</td>
<td>rs2900478</td>
<td>SLCO1B1</td>
<td>A</td>
<td>T</td>
<td>Stage 1</td>
<td>16,749</td>
<td>0.165</td>
<td>0.016</td>
<td>0.003</td>
<td>−1.6</td>
<td>2.26 × 10^{-6}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stage 2</td>
<td>7,504</td>
<td>0.164</td>
<td>0.017</td>
<td>0.006</td>
<td>−1.7</td>
<td>3.54 × 10^{-3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td>24,253</td>
<td>0.163</td>
<td>0.016</td>
<td>0.003</td>
<td>−1.6</td>
<td>1.22 × 10^{-9}</td>
</tr>
<tr>
<td>19</td>
<td>50107480</td>
<td>rs445925</td>
<td>APOE</td>
<td>A</td>
<td>G</td>
<td>Stage 1</td>
<td>13,909</td>
<td>0.098</td>
<td>−0.043</td>
<td>0.005</td>
<td>4.3</td>
<td>1.58 × 10^{-10}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stage 2</td>
<td>3,613</td>
<td>0.157</td>
<td>−0.088</td>
<td>0.011</td>
<td>8.8</td>
<td>1.41 × 10^{-15}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td>17,522</td>
<td>−0.051</td>
<td>0.005</td>
<td>5.1</td>
<td>8.52 × 10^{-29}</td>
<td></td>
</tr>
</tbody>
</table>

Chr, chromosome; SNP, single nucleotide polymorphism.

*Beta for difference between the natural log-transformed on- and off-treatment low-density lipoprotein cholesterol (LDL-C) levels adjusted for natural log-transformed off-treatment LDL-C, age, sex, and study-specific covariates. The beta reflects the fraction of differential LDL-C lowering in carriers versus non-carriers of the SNP; a negative beta indicates a better statin response (stronger LDL-C reduction), a positive beta a worse statin response. Betas and P values were generated using linear regression analysis.

†This percentage reflects the % extra LDL-C lowering in carriers versus non-carriers of the SNP.

Genomic Investigation of Statin Therapy (GIST) consortium
Combination of multiple statins dose adjusted.

SLCO1B1 Minor C Allele Associated with Methotrexate Clearance

Meta-analysis of St Jude (n=699) and COG (n=1279) patients, after we adjusted for rs4149056.

Methotrexate clearance is adjusted for age, sex, race, and treatment arm

Anything new emerging?
SLC22 and SLC47 Family Transporters


= SLC22 Family

= SLC47 Family
## Genetics of the Human OCTs and MATEs

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene</th>
<th>Location</th>
<th>Mutation</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT1</td>
<td>SLC22A1</td>
<td>Liver (sinusoidal)</td>
<td>Variety of reduced function, reduced expression and deletion variants</td>
<td>Metformin PK and PD and other emerging drugs (Level 3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intestinal Epithelial (basolateral)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCT2</td>
<td>SLC22A2</td>
<td>Kidney (proximal tubules)</td>
<td>Reduced function variants</td>
<td>Metformin PK (Level 3)</td>
</tr>
<tr>
<td>OCT3</td>
<td>SLC22A3</td>
<td>Ubiquitous</td>
<td>Reduced expression variants</td>
<td>No clinical correlates to date. (Level 3)</td>
</tr>
<tr>
<td>MATE1</td>
<td>SLC47A1</td>
<td>Kidney, Liver</td>
<td>Multiple decreased function variants</td>
<td>Metformin efficacy. (Level 3)</td>
</tr>
<tr>
<td>MATE2K</td>
<td>SLC47A2</td>
<td>Kidney</td>
<td>Reduced function variants</td>
<td>Metformin PK and efficacy (Level 2B)</td>
</tr>
</tbody>
</table>
## Drugs Transported by OCT1

<table>
<thead>
<tr>
<th>Metformin</th>
<th>Gefitinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimetidine</td>
<td>Amantadine</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Furamidine</td>
</tr>
<tr>
<td>Quinidine</td>
<td>Levodopa</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>Acyclovir</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Lamivudine</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>Sulpiride</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Ondansetron</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Tropisetron</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>O-Desmethyltramadol</td>
</tr>
<tr>
<td>Erlotinib</td>
<td></td>
</tr>
</tbody>
</table>

## DDI’s Attributed to OCT1

<table>
<thead>
<tr>
<th>Overall Effect</th>
<th>Object</th>
<th>Precipitant</th>
<th>% Change AUC</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Vivo Induction</td>
<td>metformin</td>
<td>rifampin</td>
<td>13.4</td>
<td>Cho et al. Clin Pharmal Ther 2011</td>
</tr>
<tr>
<td>&gt; 20% Effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Vivo Inhibition</td>
<td>metformin</td>
<td>trimethoprim</td>
<td>29.7</td>
<td>Muller et al. Eur J Clin Pharmcol 2015</td>
</tr>
<tr>
<td>&gt; 20% Effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Vivo Inhibition</td>
<td>metformin</td>
<td>verapamil</td>
<td>7.5 (PD effect)</td>
<td>Cho et al Br J Clin Pharm 2014</td>
</tr>
<tr>
<td>&gt; 20% Effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Condensed information from University of Washington DDI database.
Criteria for Clinically Important Transporter Polymorphisms

♦ (i) genome-wide association studies have identified them to be significantly associated with the pharmacokinetics or pharmacodynamics of one or more drugs at genome-wide level significance

♦ (ii) multiple candidate gene studies have identified significant associations between these polymorphisms and drug disposition, efficacy, or toxicity

♦ (iii) the polymorphisms have exhibited functional changes in in vitro studies

In Vitro Characterization of OCT1 Variants

- Coding region variants with both increased and decreased function.
- Reduced function and deletion variants are rare.

Expression of GFP-OCT in MDCK Cells

GFP-OCT1

GFP-OCT1-G465R

Shu et al. PNAS 2003.
Differential Effects of Variants on IC50’s for OCT1 with Metformin as Substrate

Table 4  IC50 values and the IC50 ratios for the OCT1-reference, M420del and V408M

<table>
<thead>
<tr>
<th></th>
<th>Frequency (%)</th>
<th>Reported C_max (µM)</th>
<th>Predicted C_max, portal (µM)</th>
<th>Reference IC50 (µM)</th>
<th>M420del IC50 (µM)</th>
<th>V408M IC50 (µM)</th>
<th>Ratio Ref/M420del</th>
<th>Ratio Ref/V408M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>4.9</td>
<td>0.60</td>
<td>15.05</td>
<td>0.62 (± 1.07)</td>
<td>0.09 (± 1.87)</td>
<td>0.63 (± 1.25)</td>
<td>6.84</td>
<td>0.99</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>3.2</td>
<td>0.72</td>
<td>33.0</td>
<td>6.99 (± 1.39)</td>
<td>4.70 (± 1.56)</td>
<td>4.55 (± 1.19)</td>
<td>1.49</td>
<td>1.54</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>37.0</td>
<td>0.73</td>
<td>1.41</td>
<td>199 (± 1.47)</td>
<td>85.8 (± 1.97)</td>
<td>—</td>
<td>2.32</td>
<td>—</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>21.3</td>
<td>4.49</td>
<td>12.3</td>
<td>185 (± 1.46)</td>
<td>178 (± 2.62)</td>
<td>—</td>
<td>1.04</td>
<td>—</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>20.7</td>
<td>0.13</td>
<td>9.03</td>
<td>89.0 (± 1.25)</td>
<td>26.5 (± 1.87)</td>
<td>—</td>
<td>3.36</td>
<td>—</td>
</tr>
</tbody>
</table>

*aThe treatment frequency of the drugs was derived from prescription data for 11 319 US patients treated with metformin, the type 2 diabetes drug.  
bThe reported total C_max obtained from Goodman and Gillman’s and Clarke’s isolation and identification of drugs.  
cPredicted portal vein concentration as described by Ito et al.  
dIC50 ratios between OCT1-reference and M420del/V408M.  
IC50 values were derived from concentration-dependent inhibition curves of metformin uptake.

Variants are a combination of R61C (n=4), G401S (n=3), 420del (n=1), 420del and G465R (n=3), G174S and 420del (n=1)

Table 3 Metformin pharmacokinetic parameters from healthy individuals who only carry OCT1-reference alleles (OCT1-reference) and those who carry an OCT1 variant allele

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OCT1-reference (n=8)</th>
<th>OCT1-variant (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>7.3</td>
<td>2.3</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>1.9</td>
<td>0.52</td>
</tr>
<tr>
<td>$C_{max}$ (µg/ml)</td>
<td>1.3</td>
<td>0.10</td>
</tr>
<tr>
<td>AUC$_A$ (h µg/l)</td>
<td>7,700</td>
<td>970</td>
</tr>
<tr>
<td>AUC$_B$ (h µg/l)</td>
<td>4,500</td>
<td>1,200</td>
</tr>
<tr>
<td>V/F (l)</td>
<td>2,600</td>
<td>1,800</td>
</tr>
<tr>
<td>CL/F (l/h)</td>
<td>240</td>
<td>73</td>
</tr>
<tr>
<td>CL$_R$ (l/h)</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>$f_{uo}$ (%)</td>
<td>19</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Tramadol: Interplay of CYP2D6 and OCT1 Genetics

- Tramadol is a mu-opioid receptor agonist
- Tramadol is not a substrate of OCT1
- The active desmethyl metabolite is a substrate for OCT1

• OCT expression had no effect on parent exposure
• Number of active OCT1 alleles correlated with both exposure and pupil diameter (measure of efficacy)

Effect of Number of OCT1 Active Alleles on 5HT3 Antagonists

GWAS Studies for SLC22A1

♦ No GWAS studies linking SLC22A1 to metformin PK or PD

♦ Positive GWAS studies with SLC22A1
  • Metabolite transport (isobuoyrylcarbinite)
  • Prostate Cancer

♦ Why?
  • Not important in Drug Disposition
  • SLC022A1 variants are rare
    – Not on the platform or linked to a SNP that is?
    – GWAS population not large enough?
    – Can the rare variants be combined for the GWAS?
Metformin Pharmacokinetic Pathway

Multiple genes involved in the pharmacokinetics of metformin.

GWAS Studies for SLC22A1

♦ Will this change?
  • More comprehensive arrays
  • Whole genome sequencing
  • Larger multicenter studies
  • Algorithms to combine SNPs

♦ Stay Tuned!
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Clinical Annotation Levels of Evidence

Level 1A
Annotation for a variant-drug combination in a CPIC or medical society-endorsed PGx guideline, or implemented at a PGRN site or in another major health system.

Level 1B
Annotation for a variant-drug combination where the preponderance of evidence shows an association. The association must be replicated in more than one cohort with significant p-values, and preferably will have a strong effect size.

Level 2A
Annotation for a variant-drug combination that qualifies for level 2B where the variant is within a VIP (Very Important Pharmacogene) as defined by PharmGKB. The variants in level 2A are in known pharmacogenes, so functional significance is more likely.

Level 2B
Annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated but there may be some studies that do not show statistical significance, and/or the effect size may be small.

Level 3
Annotation for a variant-drug combination based on a single significant (not yet replicated) or annotation for a variant-drug combination evaluated in multiple studies but lacking clear evidence of an association.

Level 4
Annotation based on a case report, non-significant study or in vitro, molecular or functional assay evidence only.