Recommendations for Clinical Warfarin Genotyping Allele Selection: A Joint Report of AMP and CAP

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- **Karen E. Weck**, University of North Carolina
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• **Goals**
  To develop documents/recommendations for the clinical PGx testing community by defining a minimum set of variants, or a list of “Must-Test” that should be included in genotyping assays

• **Members**
  Representatives from the clinical PGx testing community, the CDC GeT-RM Program, CPIC/PharmGKB, and CAP

• **Progress**

  **Published:**
  CYP2C19 genotyping recommendations (Pratt VM, et al. JMD, 2018;20:269-276);
  CYP2C9 genotyping recommendations (Pratt VM, et al. JMD, 2019;21:746-755);

  **In Press:**
  Warfarin sensitivity genotyping recommendations (Pratt VM, et al. in press, JMD)
Why a “Must-Test” List?

• NHGRI Genomic Medicine X: Research Directions in Pharmacogenomics Implementation
  – Call for assay standardization

• Recent GeT-RM paper (Pratt et al., 2016: no two PGx assays are testing same variants)

• Standardization of testing
  – Similar to CFTR testing

• Allele function derived from *in vitro* models may not directly translate to a clinical phenotype

• Allele function can be substrate and/or drug concentration dependent

• No clinical guidelines on how to interpret novel PGx variants

https://www.genome.gov/27568408/genomic-medicine-x-research-directions-in-pharmacogenomics-implementation/
Why NOT a “Must-Test” List?

• Targeted genotyping is a biased approach
• High throughput DNA sequencing has become more common
  – PGx genes have many challenges using NGS
• May quickly lose relevance
  – AMP PGx Working Group plans to periodically reassess and update
Defining a “Must-Test” List

Special Aim 1: *to define the key attributes of PGx alleles recommended for clinical testing*

- Framework for evaluation
  - Functional status
  - Multiethnic allele frequencies
  - Availability of reference materials (RM, CDC GeT-RM)
  - Commercially available genotyping platforms

- Started with *CYP2C19* and *CYP2C9*
Defining a “Must-Test” List

Special Aim 2: *to define a minimum set of variants that should be included in clinical PGx genotyping assays*

• Development process
  – Review of available literature, clinical guidelines, and testing resources
  – Identification of available RMs
  – Review of clinical testing currently being offered
  – Review of available quality programs
  – Identification of heterogeneity / gaps in practice
  – Discussion
  – Expert consensus recommendation/opinion development
Other Clinical Guidelines for Warfarin PGx

• Clinical dosing guidelines
  – Drug-gene pairs, with an emphasis on interpretation of genotype and phenotype, and phenotype-guided therapeutic recommendations

• CPIC, ACMG, CPDNS, DPWG (acenocoumarol and phenprocoumon)

• CPDNS: *CYP2C9*2, *3, and *VKORC1* c.-1639G>A

• CPIC warfarin dosing guideline
  – Original (2011): *CYP2C9*2, *3, and *VKORC1* c.-1639G>A
  – Updated (2017):
    • For patients without African decent: optional testing for *CYPC9*5, *6, *8, *11, and *CYP4F2*3
Variability in Clinical PGx Assays May Lead to:

- Discrepant PT survey results (e.g., CAP PGx surveys)
- Inconsistent interpretation (genotype -> haplotype -> diplotype)
- Incorrect metabolizer phenotype prediction
- Discordant therapeutic recommendations
- Variability in assays across laboratories and within assays from the same laboratory
  - An example: LDT *CYP2C9* genotyping assays from one lab
    - Version 1: *2 and *3
The Ultimate Goal

To promote standardization of PGx gene/allele testing across clinical laboratories
AMP PGx Working Group

“Two-Tier” Recommendations

• **Tier 1:**
  – A minimum or “must-test” panel of variant alleles, meeting all 3 criteria
  – Well-characterized alteration of function of the protein and/or leading to an alteration in a drug response phenotype
  – Appreciable (>1%) minor allele frequency in at least one population/ethnic group
  – RMs are publicly available

• **Tier 2:**
  – Optional extended panel of variant alleles
  – Meet at least one, but not all the criteria for inclusion in Tier 1

• **Note:**
  – Variants with unknown or uncertain function are not recommended for inclusion in clinical test panels
  – “Tag” SNPs for functional variants are not recommended
## Additional Warfarin PGx Tier 1 Alleles

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele Functional Status</th>
<th>Allele</th>
<th>Defining Functional Variant</th>
<th>HGVS genomic Nomenclature</th>
<th>HGVS cDNA Nomenclature</th>
<th>HGVS protein Nomenclature</th>
<th>Reference Material Available</th>
<th>Multiethnic Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>VKORC1</td>
<td>c.-1639G&gt;A</td>
<td>Decreased gene expression</td>
<td>rs9923231</td>
<td>NG_011564.1: g.3588G&gt;A</td>
<td>NM_024006.5: c.-1639G&gt;A</td>
<td>N/A</td>
<td>Yes</td>
<td>10-88%</td>
</tr>
</tbody>
</table>

- **VKORC1** c.-1639G>A, a promoter variant, associated with reduced expression of the warfarin target and lower dosing requirement (c.-1639A)
- Common polymorphism, in ~41-47% Caucasian and Middle Eastern, ~88% East Asian, and ~13% African, and ~15% South/Central Asian populations
- 1173C>T, rs9934438, in high LD with c.-1639G>A in most populations, “tag” SNP for functional variant, not included in either Tier 1 or 2
- 2017 CAP PT survey: 80% labs test only c.-1639G>A, 21% labs test both variants, and 4% labs do not test the c.-1639G>A

https://jmd.amjpathol.org/article/S1525-1578(20)30298-1/pdf
# Additional Warfarin PGx Tier 2 Alleles

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Allele Functional Status</th>
<th>Defining Functional Variant</th>
<th>HGVS genomic Nomenclature</th>
<th>HGVS cDNA Nomenclature</th>
<th>HGVS protein Nomenclature</th>
<th>Reference Material Available</th>
<th>Multiethnic Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP4F2</td>
<td>*3</td>
<td>Uncertain/unknown function</td>
<td>rs2108622</td>
<td>NG_007971.2: g.23454G&gt;A</td>
<td>NM_001082.4: c.1297G&gt;A</td>
<td>p.Val433Met</td>
<td>Yes</td>
<td>10-40%</td>
</tr>
<tr>
<td>VKORC1</td>
<td></td>
<td>Warfarin resistant</td>
<td>rs72547529</td>
<td>NG_011564.1: g.6557G&gt;A</td>
<td>NM_024006.5: c.196G&gt;A</td>
<td>p.Val66Met</td>
<td>No§</td>
<td>0-0.25%</td>
</tr>
<tr>
<td>VKORC1</td>
<td></td>
<td>Warfarin resistant</td>
<td>rs61742245</td>
<td>NG_011564.1: g.5332G&gt;T</td>
<td>NM_024006.5: c.106G&gt;T</td>
<td>p.Asps36 Tyr</td>
<td>No§</td>
<td>0-3.8%</td>
</tr>
<tr>
<td>2C Cluster</td>
<td></td>
<td>unknown; variant in linkage disequilibrium with warfarin effect in individuals of West African ancestry</td>
<td>rs12777823</td>
<td>NC_000010.10: g.96405502G&gt;A</td>
<td></td>
<td></td>
<td>No§</td>
<td>0-30%</td>
</tr>
</tbody>
</table>

https://jmd.amjpathol.org/article/S1525-1578(20)30298-1/pdf
**CYP4F2*3**

- rs2108622, c.1297G>A (p.Val433Met), in exon 11
- Small but significant effect on warfarin dosing
- Uncertain function: associated with reduced protein levels either due to decreased translation or increased protein degradation
- Common variant, i.e., in ~30-43% Caucasian, Middle Western, and South/Central Asian; ~22% East Asian, and ~8% African populations
- Associated with a modest increase in warfarin dosing requirements, can be beneficial for Caucasians and Asians, but not in Africans
- In CPIC dosing guideline

**Reason for Tier 2**

- Less well-characterized functional impact
- Its effect on warfarin dosing is not consistent in the general population
- Not fulfilled Tier 1 criteria “well-characterized alteration of function of the protein and/or leading to an alteration in a drug response phenotype”
• **VKORC1** c.196G>A (p.Val66Met) and c.106G>A (p.Asp36Tyr)
  – Associated with warfarin resistance or higher dose requirement
  – Relatively rare missense variants
    • c.196G>A in ~0.3% in African population
    • c.106G>A in 4% of Ashkenazi Jewish, and ~15% in Ethiopian, and ~2.5-7% of Northeast African populations
  – Not included in other clinical guidelines
  – **Reason for Tier 2**
    • RM is not available for either variant
    • May be upgraded to Tier 1 when RMs become available
  – **Rare (MAF<0.1%) warfarin resistance variants**
    • Rare, or private mutations
    • Associated with higher dose requirement, e.g., >10mg/day
    • Not widely included in genotyping panels but can be reported using sequencing methods; not recommended by AMP
PGx Tier 2 Alleles Discussion, cont.

- **CYP2C cluster rs12777823**
  - Identified by GWAS, Chr. 10 in the CYP2 cluster region, intergenic location near CYP2C18
  - Associated with reduced dose requirement in African Americans (MAF 25%), but not in European Americans (MAF 14%)
  - Common in other populations but not associated with warfarin dose requirement
  - Included in CPIC guideline, moderate recommendation only for patients with African ancestry
  - Included in clinical warfarin genotype assays by some labs
  - **Reason for Tier 2**
    - Has a role in improving dose predictions in African patients
    - Functional impact is not fully understood
    - A tag SNP for unknown functional variant in West African population
    - No RMs
Other Warfarin Pathway Genes: **GGCX** and **CALU**

- **GGCX**, gamma-glutamyl carboxylase, catalyzing the biosynthesis of vitamin K-dependent clotting factors
  - Rare GGCX variants lead to coagulation factor deficiency
  - Common variants were shown to impact warfarin dose requirements in several populations, but the data are inconsistent
- **CALU**, calumenin, functioning as chaperone of the gamma-carboxylation system
  - An intronic variant, rs339097, common in African descent (MAF 11-14%) but rare in European populations (MAF < 1%), has been associated with higher warfarin dose requirements in African Americans and Egyptians, but not in patients with European ancestry
- Not included in any clinical dosing guidelines, not recommended by AMP
AMP PGx Working Group Documents

These recommendations are intended to:

• Inform clinical laboratory professionals when designing and validating clinical PGx assays

• Promote standardization of PGx testing across different laboratories

• Serve as references for clinicians to compare and evaluate PGx testing across laboratories

• Complement other clinical guidelines, such as those issued by the Clinical Pharmacogenetics Implementation Consortium (CPIC) with focus on the interpretation and implementation of genotyping results and therapeutic recommendations for specific drugs
Feedback Needed

For the AMP PGx Two-Tier system:
1. Account for established /establishment of clinical validity?
2. Reflect appropriate clinical implementation of PGx testing given current knowledge?
3. Flexibility for testing in a variety of practice settings?
4. Place appropriate emphasis on RM availability?
5. Have you considered the “must-test” list when designing clinical PGx test?

For this warfarin recommendation:
1. Do any other genes/alleles need to be included?
2. Do you agree with the current tier assignments of warfarin genes/alleles?
3. Do you plan to implement this recommendation in your practice?

What other PGx gene(s) documents would you like to see?