CYP2C9 Genotyping Recommendations: AMP Pharmacogenomics Working Group

AMP Clinical Practice Committee

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AMP PGx Working Group

Recommendations for Clinical Pharmacogenetic Testing: Defining a Minimum Set of Variants that should be included in Genotyping assays

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- **Lari Cavallari,** University of Florida
- **Andria L. Del Tredici**, Millennium Health
- **Houda Hachad**, Translational Software
- **Yuan Ji**, Department of Pathology and ARUP Laboratories, University of Utah School of Medicine
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- **Michelle Whirl-Carrillo,** CPIC/PharmGKB representative
- **Karen E. Weck**, Departments of Pathology and Laboratory Medicine and Genetics, University of North Carolina

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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 2 PGx assays tested 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.

https://www.genome.gov/27568408/genomic-medicine-x-research-directions-in-pharmacogenomics-implementation/

Why NOT a “Must-Test” List?

- High throughput DNA sequencing has become more common
  - PGx genes have many pseudogenes
- May quickly lose relevance
  - AMP PGx Working Group plans to periodically reassess

Defining a "Must-Test" List

GOAL #1: define the key attributes of PGx alleles recommended for clinical testing

- Needed to develop a framework for evaluation
  - Functional status
  - Multiethnic allele frequencies
  - Availability of reference materials
  - Commercially available genotyping platforms
- Started with *CYP2C19*
- Other PGx genes with clinical relevance planned

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Defining a "Must-Test" List

GOAL #2: define a minimum set of variants that should be included in clinical PGx genotyping assays

- Development process
  - Review of available literature and testing resources
  - Identification of available reference materials
  - Review of clinical testing currently being offered
  - Review of available quality programs
  - Identification of heterogeneity / gaps in practice
  - Discussion
  - Expert consensus recommendation/opinion development

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Proposed system

- **Tier 1**
  - Minimum “must-test” set
  - Well-characterized alteration of activity that has been shown to have an effect on drug response and for which the functional variant is known
  - Appreciable (≥1%) minor allele frequency in a population
  - Available reference materials
- **Tier 2**
  - Extended panel
  - Meet at least 1 but, not all of the criteria for inclusion in Tier 1

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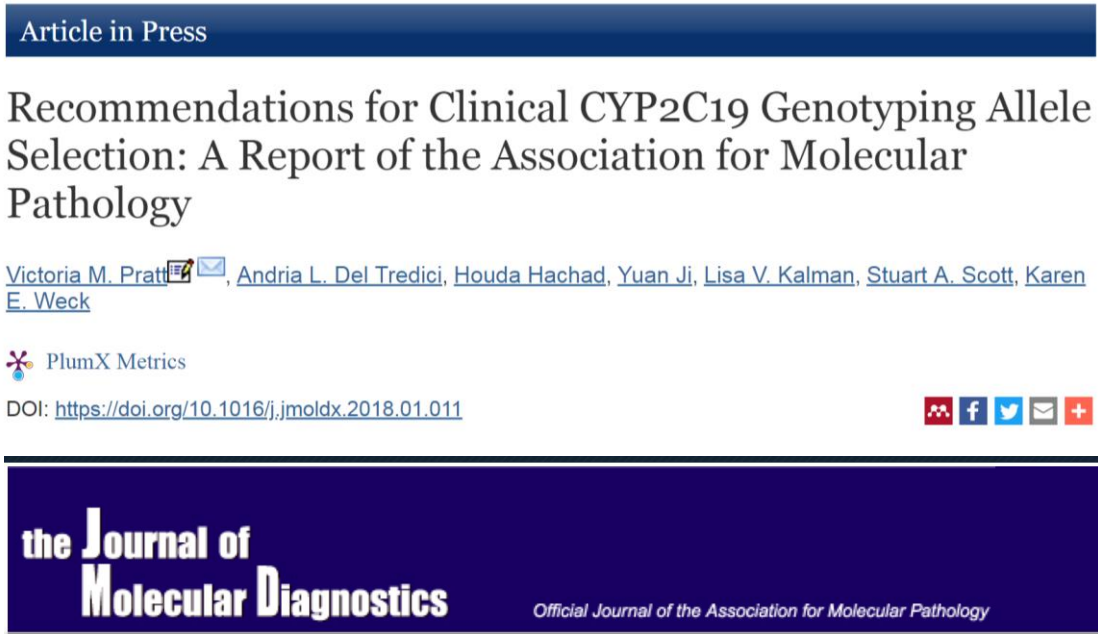
- First deliverable: consensus expert opinion recommendations for clinical *CYP2C19* testing



Article in Press

Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology

Victoria M. Pratt, Andria L. Del Tredici, Houda Hachad, Yuan Ji, Lisa V. Kalman, Stuart A. Scott, Karen E. Weck

PlumX Metrics

DOI: https://doi.org/10.1016/j.jmoldx.2018.01.011

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Official Journal of the Association for Molecular Pathology

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AMP PGx Working Group
Proposed system

- **Tier 1**
  - Minimum “must-test” set
  - Well-characterized alteration of activity that has been shown to have an effect on drug response and for which the functional variant is known
  - Appreciable (≥1%) minor allele frequency in a patient population
  - Available reference materials
- **Tier 2**
  - Extended panel
  - Meet at least 1 but, not all of the criteria for inclusion in Tier 1
- **Other**
  - Variants with unknown or uncertain function are not recommended for inclusion in clinical test panels

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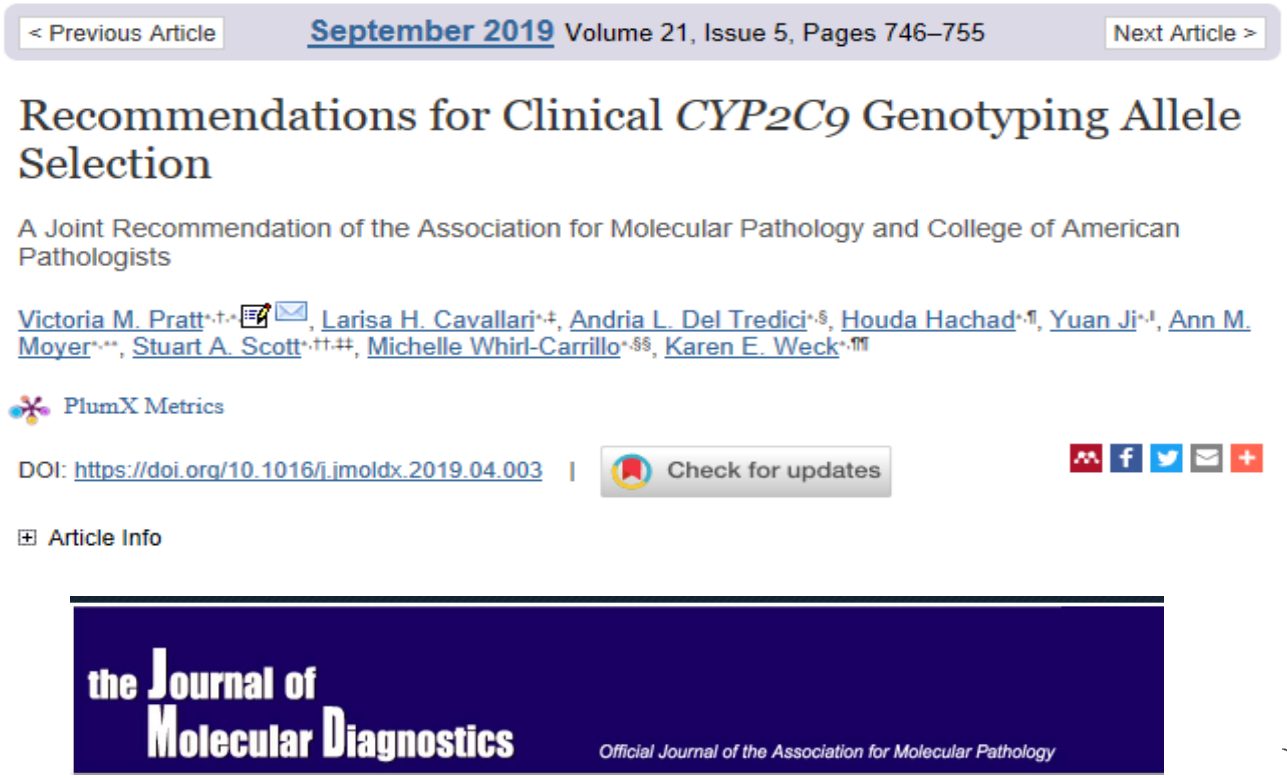
- 2nd deliverable: consensus expert opinion recommendations for clinical *CYP2C9* testing



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Recommendations for Clinical *CYP2C9* Genotyping Allele Selection

A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists

Victoria M. Pratt[*,†,*], Larisa H. Cavallari[*,‡], Andria L. Del Tredici[*,§], Houda Hachad[*,¶], Yuan Ji[*,‖], Ann M. Moyer[*,**], Stuart A. Scott[*,††,‡‡], Michelle Whirl-Carrillo[*,§§], Karen E. Weck[*,¶¶]

PlumX Metrics

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Article Info

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CYP2C9 Medications
FDA labeling

Generic Name	Trade Name (s)	Label Date	Link to FDA Label
Celecoxib	Celebrex	5/9/2016	https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/020998s048lbl.pdf
Dronabinol	Marinol	8/28/2017	https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/018651s029lbl.pdf
Flurbiprofen	Ansaid	5/9/2016	https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018766s020lbl.pdf
Fosphenytoin	Cerebyx	10/31/2017	https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/020450s037s038lbl.pdf
Lesinurad	Zurampic	12/22/2015	https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/207988lbl.pdf
Phenytoin	Dilantin	12/22/2017	https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/084349s081s082s084lbl.pdf
Piroxicam	Feldene	5/9/2016	https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018147s044lbl.pdf
Warfarin	Coumadin	8/14/2017	https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/009218s118lbl.pdf

AMP Guidelines for *CYP2C9* clinical testing – Tier 1

Allele	Allele Functional Status†	Defining Functional Variant	HGVS Nomenclature: NM_000771.3	HGVS Nomenclature: NG_008385.1§	Reference Material Available	Multiethnic Allele Frequency
*2¶	Decreased function	rs1799853	c.430C>T, p.Arg144Cys	g.8633C>T	Yes	0-12%
*3‡	Decreased function	rs1057910	c.1075A>C, p.Ile359Leu	g.47639A>C	Yes	1-11%
*5	Decreased function	rs28371686	c.1080C>G, p.Asp360Glu	g.47644C>G	Yes	0-1%
*6	No function	rs9332131	c.818del, p.Lys273Argfs*34	g.15625delA	Yes	0-1%
*8	Decreased function	rs7900194	c.449G>A, p.Arg150His	g.8652G>A	Yes	0-5%
*11	Decreased function	rs28371685	c.1003C>T, p.Arg335Trp	g.47567C>T	Yes	0-2%

† Citations for assignment of function can be found at https://www.pharmvar.org/gene/CYP2C9, last accessed 8/15/2018 § CYP2C9 RefSeqGene; ¶ Note that the defining variant of the *35 allele (c.374G>T, p.Arg125Leu) is likely in linkage disequilibrium with the defining *2 variant (c.430C>T, p.Arg144Cys)). ‡ Note that the defining *18 variant of the allele (c.1190A>C, p.Asp397Ala, rs72558193) is likely in linkage disequilibrium with the defining variant of *3 variant (c.1075A>C, p.Ile359Leu, rs1057910)

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African ancestry alleles

- *5, *6, *8, *11
- Increases detection by 10%
- WG felt important to include

CYP2C9 Allele	African	Other	Latino	South Asian	European	East Asian	Finnish
*2	2.354000%	9.471000%	6.600000%	4.603000%	12.680000%	0.034790%	11.700000%
*3	1.259000%	6.608000%	3.580000%	11.310000%	6.882000%	3.379000%	5.633000%
*5	1.268000%	0.220300%	0.034860%	0.000000%	0.004497%	0.000000%	0.000000%
*6	1.049000%	0.000000%	0.035090%	0.000000%	0.003010%	0.000000%	0.000000%
*8	5.603000%	0.000000%	0.216900%	0.060560%	0.025470%	0.011590%	0.000000%
*11	2.143000%	0.330400%	0.156700%	0.193800%	0.218800%	0.011570%	0.544500%

http://exac.broadinstitute.org/

AMP Guidelines for *CYP2C9* clinical testing - Tier 2

Allele	Allele Functional Status†	Defining Functional Variant	HGVS Nomenclature: NM_000771.3	HGVS Nomenclature: NG_008385.1§	Reference Material Available	Multiethnic Allele Frequency
*12	Decreased function	rs9332239	c.1465C>T, p.Pro489Ser	g.55363C>T	Yes	0-0.3%
*13	Decreased function	rs72558187	c.269T>C, p.Leu90Pro	g.8301T>C	No	0-0.2%
*15	No function	rs72558190	c.485C>A, p.Ser162*	g.14125C>A	No	0-0.01%

§ CYP2C9 RefSeqGene; forward relative to chromosome), † https://www.pharmvar.org/gene/CYP2C9, last accessed 8/15/2018

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CYP2C9: one of the most tested PGx genes

- >50 laboratories listed in Genetic Testing Registry https://www.ncbi.nlm.nih.gov/gtr/
- Large variation in allele selection of LDPs across labs (targeted genotyping, full sequencing, ± deletion/duplication analysis)
- Large variation in commercial PGx genotyping platforms
- The majority of LDPs/tests currently do not include all the Tier 1 alleles, indicating that these recommendations may be more difficult to implement

	Affymetrix PharmacoScan (RUO)†	Agena Biosciences iPLEX ADME (RUO)‡	Autogenomics INFINITI§ (CE-marked)	GenMark eSensor (FDA-cleared)¶	BioFire Defense analyte-specific reagents)	Thermo Fisher OpenArray (V, RUO)†	TrimGen (FDA-cleared)
CYP2C9 Alleles	*2, *3, *4, *5, *5, *8, *9, *10, *11, *13, *15, *16, *17, *18, *19, *20, *21, *23, *24, *25, *26, *29, *30, *31, *32, *34, *36, *37, *38, *39, *40, *42, *43, *44, *45, *46, *47, *48, *49, *50, *51, *52, *53, *54, *55, *56, *57, *58	*1B, *1C, *1D, *2, *2C *3A, *3B,*4, *5, *5, *8, *9, *10, *11A, *11B, *13, *15, *16, *17, *18, *19, *20, *21, *22, *23, *24, *25, *26, *27, *28, *29, *30, *31, *32, *33, *34	*2, *3, *4, *5, *6, *11	*2, *3	*2, *3	variable	*2, *3

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AMP PGx Working Group Summary Genotyping Recommendations

These recommendations are intended to

- Inform clinical laboratory professionals when designing and validating clinical PGx assays
- Promote standardization of PGx testing across different laboratories
- Complement other clinical guidelines, such as those issued by the Clinical Pharmacogenetic implementation consortium (CPIC)
  - *which primarily focus on the interpretation of genotyping results and therapeutic recommendations for specific drugs*

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We want to hear from you!

- Does the proposed two tier system
  - Account for established /establishment of clinical utility?
  - Reflect appropriate clinical implementation of PGx testing given current knowledge?
  - Flexibility for testing in a variety of practice settings?
  - Place appropriate emphasis on reference material availability?
- Other alleles that should be included?
- Do you agree with the current tier assignments for the listed alleles? If not, why not?

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A Special Thanks

- **AMP Clinical Practice Committee**
- **Mrudula Pullambhatla**
- **Robyn Temple-Smolkin**

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