More is Better, right? Panel vs. Targeted Testing

V.M. Pratt, Ph.D, FACMG

Adjunct Professor, Medical and Molecular Genetics

INDIANA UNIVERSITY

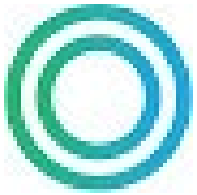
Disclosures

- Employee, Optum Genomics

Objectives

- Understand the different test panels
- Differentiate gene coverage
- Describe standardization efforts

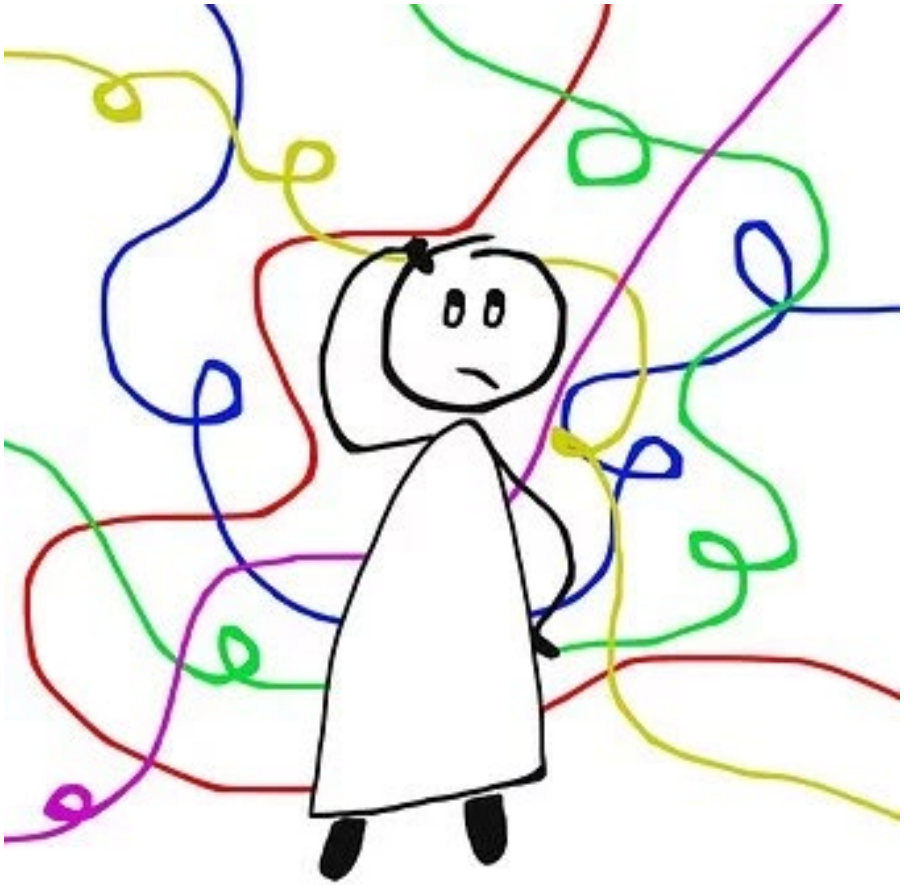
There are lots of lab tests!



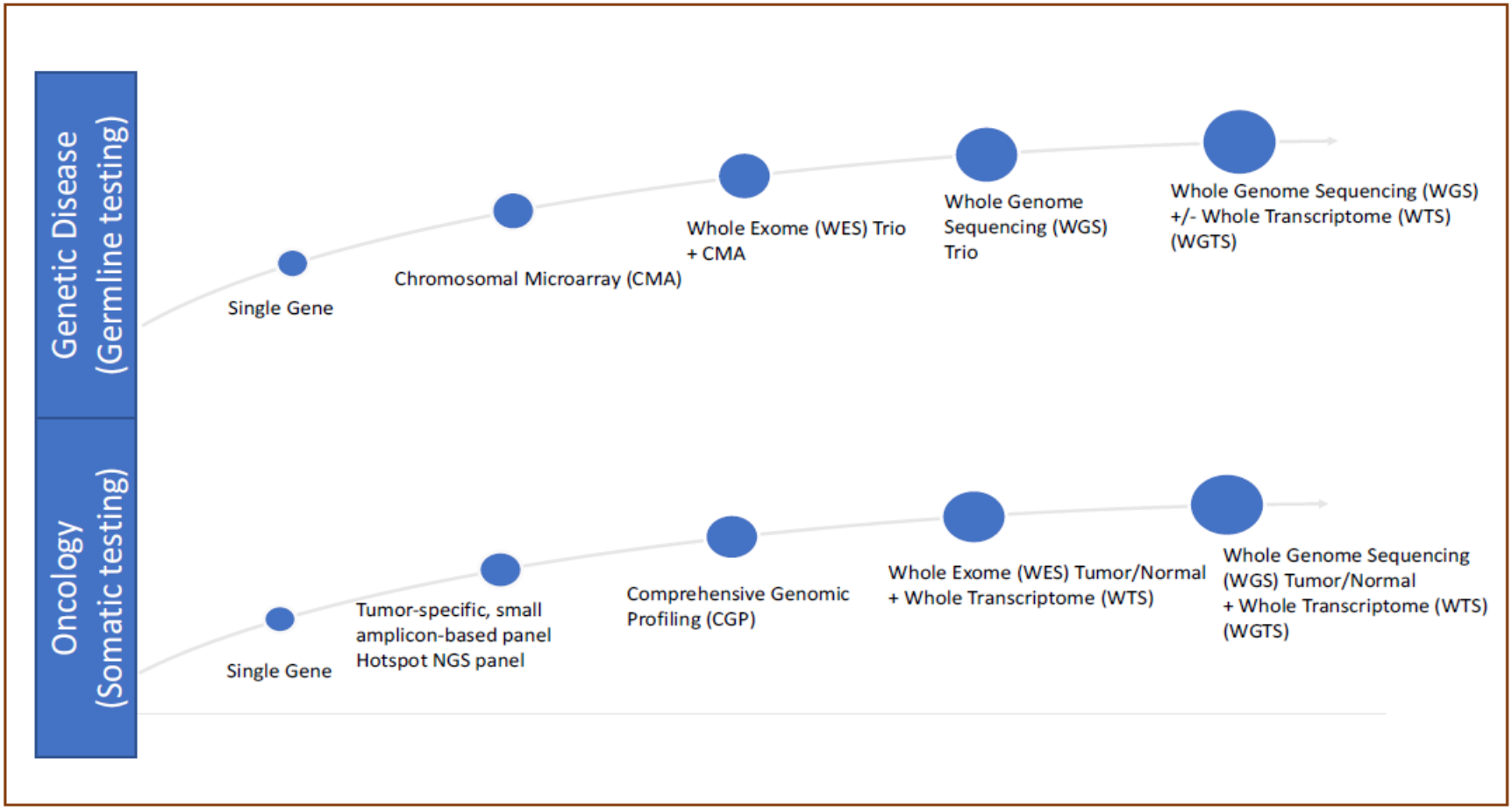
Physicians are introduced to many new genetic tests

Coldbrook Insurance | Physician's Practice
Imatest Master | Imatest

Which test is right for my patients?



Testing (r)evolution



Testing (r)evolution

Targeted

Whole “omic” approach

Risk Icon On Speedometer High Risk Meter Vector Stock Illustration Stock Illustration - Download Image Now - iStock (istockphoto.com)
https://www.alamy.com/stock-photo-young-strong-woman-warrior-with-big-guns-in-dramatic-urban-night-scene-145283794.html

PANEL (TARGETED) TESTING

Risk Icon On Speedometer High Risk Meter Vector Stock Illustration Stock Illustration - Download Image Now - iStock (istockphoto.com)

Different (PGx) panels include different genes

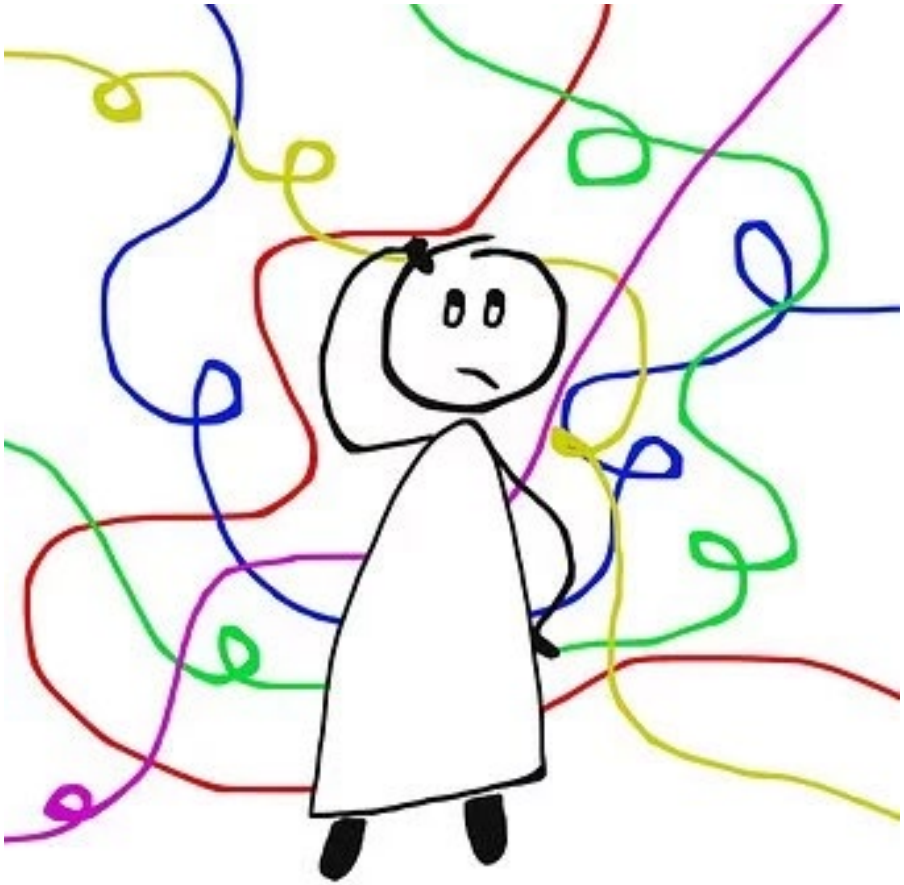
- *CYP1A2*
- *CYP2B6*
- *CYP2C19*
- *CYP2C9*
- *CYP2D6*
- *CYP3A4*
- *HLA-A*
- *HLA-B*
- *HTR2A*
- *SLC64A*
- *UGT1A4*
- *UGT2B15*

- *CYP1A2*
- *CYP2B6*
- *CYP2C Cluster*
- *CYP2C19*
- *CYP2C9*
- *CYP2D6*
- *CYP3A4*
- *CYP3A5*
- *CYP4F2*
- *COMT*
- *DPYD*
- *F2*
- *F5*
- *GRIK4*
- *HLA-A*
- *HLA-B*
- *HTR2A*
- *HTRC2*
- *IL28B (IFNL3)*
- *MTHFR*
- *NUDT15*
- *UGT1A1*
- *VKORC1*

- *BDNF*
- *COMT*
- *CYP1A2*
- *CYP2B6*
- *CYP2C19*
- *CYP2C9*
- *CYP2D6*
- *CYP3A4*
- *CYP3A5*
- *HLA-A*
- *HTR2A*
- *MC4R*
- *MTHFR*
- *SLC64A*
- *UGT2B15*

- *CYP2B6*
- *CYP2C Cluster*
- *CYP2C19*
- *CYP2C9*
- *CYP2D6*
- *CYP3A4*
- *CYP3A5*
- *CYP4F2*
- *DPYD*
- *NUDT15*
- *SCLO1B1*
- *TPMT*
- *VKORC1*

What genes should be included for a clinical indication?



BDNF

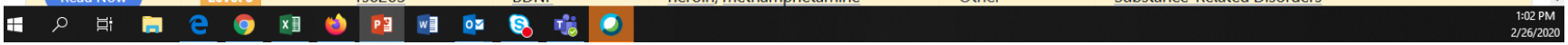
	LEVEL	VARIANT	GENE	MOLECULE	TYPE	PHENOTYPE
Read Now	Level 3	rs7124442	BDNF	citalopram	Efficacy	Depressive Disorder, Major
Read Now	Level 3	rs6265	BDNF	Analgesics, Antiinflammatory agents, non-steroids, Ergot alkaloids, opioids, sumatriptan	Other	
Read Now	Level 3	rs6265	BDNF	paroxetine	Efficacy	Depressive Disorder, Major
Read Now	Level 3	rs7934165	BDNF	methadone	Dosage	Heroin Dependence, Opioid-Related Disorders
Read Now	Level 3	rs962369	BDNF	escitalopram, nortriptyline	Toxicity/ADR	Depressive Disorder, Major
Read Now	Level 3	rs10835210	BDNF	methadone	Dosage	Heroin Dependence, Opioid-Related Disorders
Read Now	Level 3	rs7103411	BDNF	citalopram	Efficacy	Depressive Disorder, Major
Read Now	Level 3	rs11030104	BDNF	antipsychotics	Efficacy	Schizophrenia
Read Now	Level 3	rs6265	BDNF	antidepressants, citalopram, paroxetine	Efficacy	Depressive Disorder
Read Now	Level 3	rs6265	BDNF	heroin, methamphetamine	Other	Substance-Related Disorders

https://www.pharmgkb.org/gene/PA31891/clinicalAnnotation

BDNF

	LEVEL	VARIANT	GENE	MOLECULE	TYPE	PHENOTYPE
Read Now	Level 3	rs7124442	BDNF	citalopram	Efficacy	Depressive Disorder, Major
Read Now	Level 3					
Read Now	Level 3					
Read Now	Level 3					id-Related Disorders
Read Now	Level 3	rs962369	BDNF	escitalopram, nortriptyline	Toxicity/ADR	Depressive Disorder, Major
Read Now	Level 3	rs10835210	BDNF	methadone	Dosage	Heroin Dependence, Opioid-Related Disorders
Read Now	Level 3	rs7103411	BDNF	citalopram	Efficacy	Depressive Disorder, Major
Read Now	Level 3	rs11030104	BDNF	antipsychotics	Efficacy	Schizophrenia
Read Now	Level 3	rs6265	BDNF	antidepressants, citalopram, paroxetine	Efficacy	Depressive Disorder
Read Now	Level 3	rs6265	BDNF	heroin, methamphetamine	Other	Substance-Related Disorders

Is this gene for research?



https://www.pharmgkb.org/gene/PA31891/clinicalAnnotation

Evidence-based gene selection

2/3 had limited or no evidence

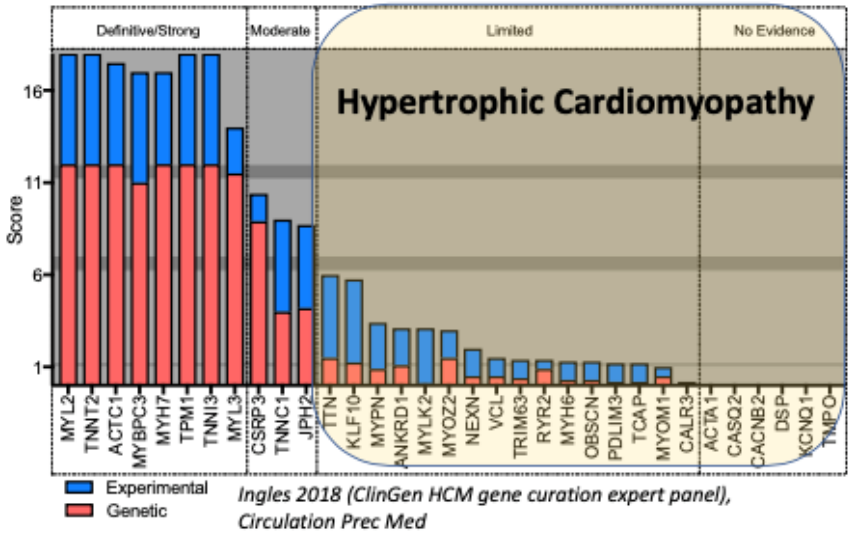
Am J Hum Genet. 2017 Jun 1;100(6):895-906. doi: 10.1016/j.ajhg.2017.04.015. Epub 2017 May 25.

Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource.

Strande NT[1], Riggs ER[2], Buchanan AH[3], Ceyhan-Birsoy O[4], DiStefano M[5], Dwight SS[6], Goldstein J[1], Ghosh R[7], Seifert BA[1], Sneddon TP[6], Wright MW[6], Milko LV[1], Cherry JM[6], Giovanni MA[3], Murray MF[3], O'Daniel JM[1], Ramos EM[8], Santani AB[9], Scott AF[10], Plon SE[7], Rehm HL[4], Martin CL[11], Berg JS[12].

Assertion criteria	Genetic Evidence (0-12 points)	Experimental Evidence (0-6 points)	Total Points (0-18)	Replication Over Time (Y/N)
Description	Case-level, family segregation, or case-control data that support the gene-disease association	Gene-level experimental evidence that support the gene-disease association	Sum of Genetic & Experimental Evidence	> 2 pubs w/ convincing evidence over time (>3 yrs)
Assigned Points	4.5	1.5	5.5	N
CALCULATED CLASSIFICATION		LIMITED	1-6	
		MODERATE	7-11	
		STRONG	12-18	
		DEFINITIVE	12-18 AND replication over time	

Categories express strength (validity) of gene-disease association



Ingles 2018 (ClinGen HCM gene curation expert panel), Circulation Prec Med

Ingles J, Goldstein J, *et al.* Evaluating the Clinical Validity of Hypertrophic Cardiomyopathy Genes. Circ Genom Precis Med. 2019 Feb;12(2):e002460. doi: 10.1161/CIRCGEN.119.002460. PMID: 30681346; PMCID: PMC6410971.

Guidelines/Reviews

- National Comprehensive Cancer Network (https://www.nccn.org/)
- Clinical Pharmacogenetics Implementation Consortium (https://cpicpgx.org/)
- GeneReviews® (https://www.ncbi.nlm.nih.gov/books/NBK1116/)

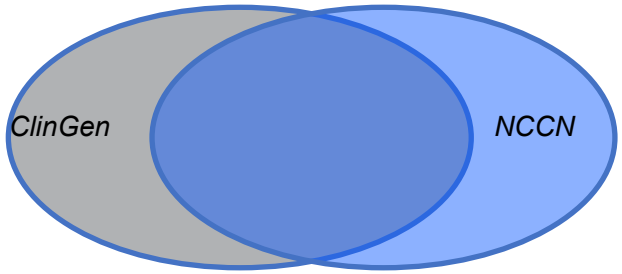
- Clinical Genome Resource (ClinGen) (Welcome to ClinGen (clinicalgenome.org)

How does one resolve discrepancies in evidence?

ClinGen vs NCCN

Cancer	Gene	ClinGen	NCCN
Breast/ovarian	*PALB2*	Moderate (2017)	Strong (2022)
Colon	*GREM1*	Strong (2016)	Not well-established (2022)

Hereditary cancer only 46 genes overlap between ClinGen (176 total curated) and NCCN (total 65 curated). ~70% agreement between ClinGen and NCCN.

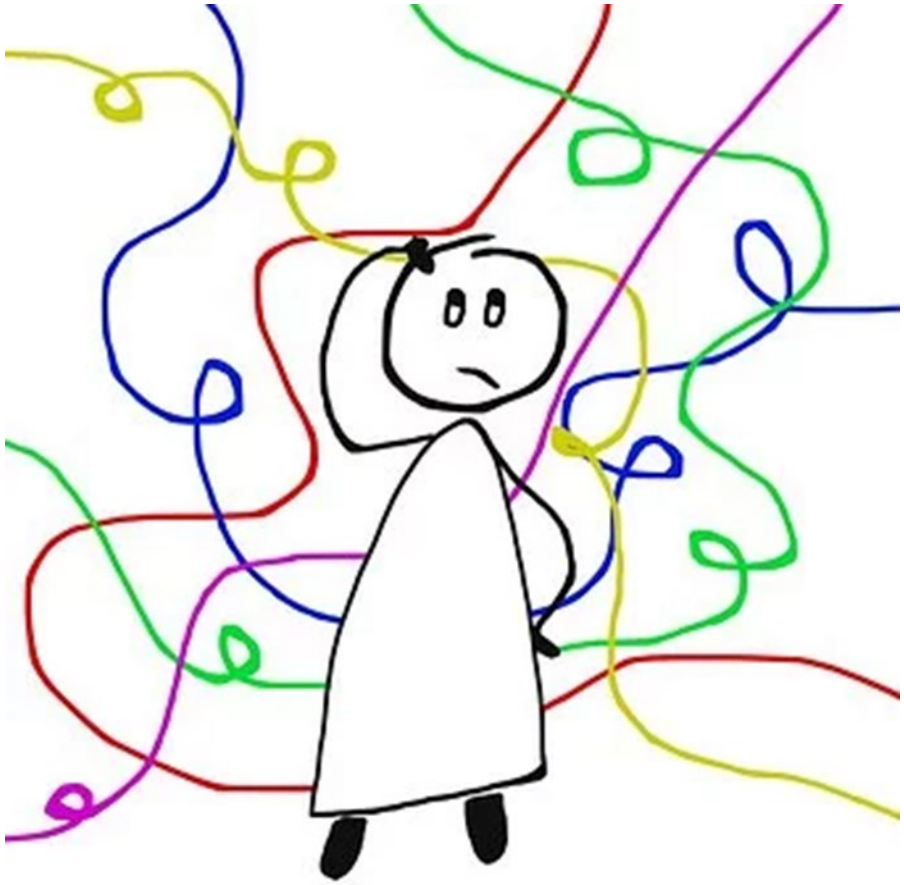


Use the union or the intersection?

- ClinGen and NCCN
- ClinGen or NCCN
- ClinGen only
- NCCN only

Are there other authoritative practice guidelines?

What variants should be tested for gene(s)?



AMP PGx Working Group

- **Victoria M. Pratt** (Chair), Optum Genomics
- **Karen E. Weck** (Co-Chair), University of North Carolina
- **Larisa H. Cavallari,** University of Florida
- **Makenzie Fulmer,** ARUP Laboratories and University of Utah School of Medicine, Junior member
- **Andrea Gaedigk,** Children's Mercy Kansas City
- **Houda Hachad**, AccessDx Laboratory
- **Yuan Ji**, ARUP Laboratories and University of Utah School of Medicine
- **Lisa V. Kalman**, Division of Laboratory Systems, Centers for Disease Control and Prevention
- **Reynold C. Ly,** Indiana University
- **Ann M. Moyer,** Mayo Clinic, CAP representative
- **Stuart A. Scott**, Stanford University Medical Center
- **Ron van Schaik,** Erasmus MC University Medical Center, ESPT and DPWG representative
- **Michelle Whirl-Carrillo,** Stanford University, CPIC/PharmGKB

PGx Genotyping Recommendations are Needed

GOAL: To promote standardization of PGx allele testing across clinical laboratories

- Inconsistent interpretation can lead to discordant therapeutic recommendations
- Publications show lack of consistency in alleles included in commercial platforms and clinical tests
- **Genomic Medicine X: Research Directions in Pharmacogenomics Implementation**

  NHGRI Meeting in 2017 - Call for assay standardization

PGx Genotyping Recommendations are Needed

- **How do we reduce the variability?**

  Option 1: Test all known alleles

  - Not practical – for *CYP2D6*, there are currently >145 alleles/sub-alleles!!!

  Option 2: Sequence instead of targeted genotyping

  - Likely coming in the future
  - Current state: Pharmacogenes are technically challenging by short-read NGS chemistry
    - Pseudogenes, homologous gene families, duplications, deletions, deeply intronic variants
    - Challenges in reporting genotypes
      - » What do you put on the clinical report when you otherwise have a *CYP2D6*2/*4,* but also see a rare variant that does not have a corresponding star allele?
    - Challenges in interpreting rare variants/alleles

  Option 3: Why not use a similar to ACMG recommendations for *CFTR* testing?

  - Define a minimum set of variants based on multiethnic allele frequency in order to optimize diagnostic test rate

AMP PGx Working Group: Expert consensus recommendation/opinion development

- **Tier 1 -** Minimum "must-test" alleles
  - Well-characterized effect on the function of the protein and/or gene expression
  - Appreciable minor allele frequency in a patient population
  - Available reference materials
  - Technical feasibility to detect variant in a clinical laboratory (NEW requirement for *CYP2D6*)
- **Tier 2 -** Extended panel
  - Meet at least one but not all the criteria for inclusion in Tier 1
- **Other**
  - Variants with unknown or uncertain function are not recommended for inclusion in clinical test panels

AMP PGx Working Group - *CYP2C19*

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

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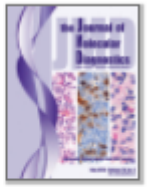
The Journal of Molecular Diagnostics

Volume 20, Issue 3, May 2018, Pages 269-276



Special article

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 *, ‡‡, §§

Tier 1 *CYP2C19* allele recommendations

Table 3

CYP2C19 Tier 1 variant alleles.

Allele	Allele Functional Status †	Defining Functional Variant	HGVS Nomenclature: NM_000769.2	HGVS Nomenclature: NG_008384.2 ‡	Reference Material Available *	Multiethnic Allele Frequency
*2§	No function	rs4244285	c.681G>A	g.24154G>A	Yes	12-54%
*3	No function	rs4986893	c.636G>A	g.22948G>A	Yes	0.3-15%
*17	Increased function	rs12248560	c.-806C>T	g.4195C>T	Yes	4-21%

- Together these 3 variants account for 40% to > 90% of the currently defined alleles in most racial and ethnic groups.
- Many of the clinical correlative studies have focused on these alleles

* ***Centers for Disease Control and Prevention GeT-RM Program*** http://wwwn.cdc.gov/clia/Resources/GetRM/

Pratt, V.M., et al. Characterization of 107 genomic DNA reference materials for CYP2D6, CYP2C19, CYP2C9, VKORC1, and UGT1A1: a GeT-RM and Association for Molecular Pathology collaborative project. J Mol Diagn. 2010; 12: 835–846

Tier 2 *CYP2C19* allele recommendations

Table 4

CYP2C19 Tier 2 variant alleles.

Allele	Allele Functional Status	Defining Variant(s)	HGVS Nomenclature: NM_000769.2	HGVS Nomenclature: NG_008384.2 †	Reference Material Available	Multiethnic Allele Frequency ‡
*4A	No function	rs28399504	c.1A>G	g.5001A>G	Yes	0.1-0.3%
*4B	No function	rs28399504; rs12248560	c.[-806C>T; 1A>G]	g.[4195C>T;5001A>G]	Yes	0-0.2%
*5	No function	rs56337013	c.1297C>T	g.95033C>T	No	0%
*6	No function	rs72552267	c.395G>A	g.17748G>A	Yes	0-0.1%
*7	No function	rs72558186	c.819+2T>A	g.24294T>A	No	0%
*8	No function	rs41291556	c.358T>C	g.17711T>C	Yes	0.1-0.3%
*9	Decreased function	rs17884712	c.431G>A	g.17784G>A	Yes	0.1-4.2%
*10	Decreased function	rs6413438	c.680C>T	g.24153C>T	Yes	0.1-6%
*35‡	No function	rs12769205	c.332-23A>G	g.17662A>G	No	0.8-3.1%

MAF < 0.5%

Intermediate function, Clinical relevance less well-defined

**35 recently defined, not as well characterized, linked to *2 variant*

- *4B = *4 (no function) and *17 (increased function) SNPs in same haplotype (in cis)
- *10 = adjacent to *2 variant, may cause interference with *2 genotyping assays when present

AMP PGx Working Group - *CYP2C9*

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

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The Journal of Molecular Diagnostics

Volume 21, Issue 5, September 2019, Pages 746-755

Special article

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 *, ¶¶

Tier 1 *CYP2C9* Alleles

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)

Tier 2 *CYP2C9* Alleles

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*

- ***12, *13, and *15: all decreased or no function alleles**
- **All have low minor allele frequencies (<0.5%)**
- ***13 and *15 currently lack RMs**

AMP PGx Working Group - Warfarin

- Third deliverable: consensus expert opinion recommendations for clinical warfarin testing

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The Journal of Molecular Diagnostics
Volume 22, Issue 7, July 2020, Pages 847-859

Special article

Recommendations for Clinical Warfarin Genotyping Allele Selection: A Report of the Association for Molecular Pathology and the College of American Pathologists

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

Tier 1 Warfarin PGx Alleles

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

- *VKORC1*2* 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" variant 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 dot not test the c.-1639G>A

Tier 2 Warfarin PGx Alleles

Gene	Allele	Allele Functional Status	Defining Functional Variant	HGVS genomic Nomenclature	HGVS cDNA Nomenclature	HGVS protein Nomenclature	Reference Material Available	Multiethnic Allele Frequency
CYP4F2	*3	Uncertain/unknown function	rs2108622	NG_007971.2: g.23454G>A	NM_001082.4: c.1297G>A	p.Val433Met	Yes	10-40%
VKORC1		Warfarin resistant	rs72547529	NG_011564.1: g.6557G>A	NM_024006.5: c.196G>A	p.Val66Met	No §	0-0.25%
VKORC1		Warfarin resistant	rs61742245	NG_011564.1: g.5332G>T	NM_024006.5: c.106G>T	p.Asp36Tyr	No §	0-3.8%
2C Cluster		unknown; variant in linkage disequilibrium with warfarin effect in individuals of West African ancestry	rs12777823	NC_000010.10: g.96405502G>A			No §	0-30%

AMP PGx Working Group *CYP2D6*

- 4th deliverable: consensus expert opinion recommendations for clinical CYP2D6 testing

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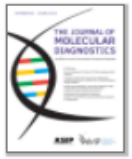
The Journal of Molecular Diagnostics
Volume 23, Issue 9, September 2021, Pages 1047-1064



Special article

Recommendations for Clinical *CYP2D6* Genotyping Allele Selection: A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy

Victoria M. Pratt *, †, ✉, Larisa H. Cavallari *, ‡, Andria L. Del Tredici *, §, Andrea Gaedigk *, ¶, Houda Hachad *, ‖, Yuan Ji *, **, Lisa V. Kalman *, ††, Reynold C. Ly *, ‡‡, Ann M. Moyer *, §§, Stuart A. Scott *, ¶¶, ‖‖, R.H.N. van Schaik *, ***, †††, Michelle Whirl-Carrillo *, ‡‡‡, Karen E. Weck *, §§§

AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686				242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097				945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655				084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656				176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs11358				692G>A, 611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C	g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C	g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

Included because

- c.886C>T (2850C>T) and c.1457G>C (4180G>C) are present in other haplotypes
- Often interrogated by labs to differentiate haplotypes

AMP Guidelines for *CYP2D6* clinical testing – Tier 1

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C	g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9-29.5%
*3	No function	rs35742686	2549delA	2550delA	g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1-1.6%
*4	No function	rs3892097	1846G>A	1847G>A	g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5-18.5%
*5	No function	*CYP2D6* full gene deletion							Yes	1.6-5.4%
*6	No function	rs5030655	1707delT	1708delT	g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0-1.1%
*9	Decreased function	rs5030656	2615delAAG	2616delAAG	g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0-2.8%
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C	g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4-43.6%
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C	g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1-19.3%
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, g.8203G>A, g.42127608C>T				p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-12.1%
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C					N/A (Splicing Defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8-15.4%
xN	variable, depending the duplicated alleles	duplications							Yes	variable

- Duplications may be detected by a single probe
  - May not always distinguish between duplications and hybrid alleles
  - Functional effect depends on allele duplicated
    - Single or duplicated *4 allele has no activity
    - Duplicated *1 has > activity than single *1

AMP Guidelines for *CYP2D6* clinical testing – Tier 2

Allele	Allele Functional Status assigned by CPIC†	Core Variant(s)‖	Legacy Nomenclature (M33388) ATG Start*‡	RefSeqGene LRG_303 (NG_008376.4) ATG Start*	RefSeqGene LRG_303 (NG_008376.4)	HGVS Genomic Nomenclature (GRCh38) (NC_000022.11)	HGVS cDNA Nomenclature LRG_303 (NM_000106.6*)	HGVS Protein Nomenclature: LRG_303 (NP_000097.3)	Reference Material Available	Multiethnic Allele Frequency
*7	No function	rs5030867	2935A>C	2936A>C	g.7955A>C			is324Pro	Yes	0-0.6%
*8	No function	rs5030865, rs16947, rs1135840	1758G>T, 2850C>T, 4180G>C	1759G>T, 2851C>T, 4181G>C	g.6778G>T, g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.1457G>C	ly169Ter, p.Arg296Cys, p.Ser486Thr	No	0-0.1%
*12	No function	rs5030862, rs16947, rs1135840	124G>A, 2850C>T, 4180G>C	124G>A, 2851C>T, 4181G>C	g.5143G>A, g.7870 g.9200G>C			p.Gly42Arg, p.Arg296Cys, p.Ser486Thr	No	0-1.7%
*14	Decreased function	rs5030865, rs16947, rs1135840	1758G>A, 2850C>T, 4180G>C	1759G>A, 2851C>T, 4181G>C	g.6778G>A, g.7870 g.9200G>C			p.Gly169Arg, p.Arg296Cys, p.Ser486Thr	Yes	0-0.3%
*15	No function	rs774671100	137_138insT	137_138insT	g.5156dup			p.Leu47fs	Yes	0-0.6%
*21	No function	rs72549352, rs16947, rs1135840	2579_2580insC, 2850C>T, 4180G>C	2580_2581insC, 2851C>T, 4181G>C	g.7599dup, g.7870 g.9200G>C	g.42126611C>G	c.1457G>C	p.Arg269fs, p.Arg296Cys, p.Ser486Thr	Yes	0-0.4%
*31	No function	rs267608319, rs16947, rs1135840	4042G>A, 2850C>T, 4180G>C	4043G>A, 2851C>T, 4181G>C	g.9062G>A, g.7870C>T, g.9200G>C	g.42126749C>T, g.42127941G>A, g.42126611C>G	c.1319G>A, c.886C>T, c.1457G>C	p.Arg440His, p.Arg296Cys, p.Ser486Thr	Yes	0-0.8%
*40	No function	rs72549356, rs28371706, rs16947, rs1135840	1863_1864ins TTTCGCCCCTTTCGCCCC, 1023C>T, 2850C>T, 4180G>C	1864_1865ins TTTCGCCCCTTTCGCCCC, 1022C>T, 2851C>T, 4181G>C	g.6875_6885 TTTCGCCCC[3], g.6041C>T, g.7870C>T, g.9200G>C	g.42126934_42126942AAAG GGGCG[3], g.42129770G>A, g.42127941G>A, g.42126611C>G	c.514_522 TTTCGCCCC[3], c.320C>T, c.886C>T, c.1457G>C	p.172_174[3], p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	0-1.3%
*42	No function					g.42127532_42127533dup,		p.Gln364fs, p.Arg296Cys, p.Ser486Thr	No	0-0.5%
*49	Decreased function							p.Phe120Ile, p.Pro34Ser, p.Ser486Thr	No	0-1.1%
*56	No function	rs72549347, rs1135840	3201C>T, 4180G>C	3202C>T, 4181G>C	g.8221C>T, g.9200G>C	g.42127590G>A, g.42126611C>G	c.1030C>T, c.1457G>C	p.Arg344Ter, p.Ser486Thr	Yes	0-0.2%
*59	Decreased function	rs79292917, rs16947, rs1135840	2939G>A, 2850C>T, 4180G>C	2940G>A, 2851C>T, 4181G>C	g.7959G>A, g.7870C>T, g.9200G>C	g.42127852C>T, g.42127941G>A, g.42126611C>G	c.975G>A, c.886C>T, c.1457G>C	p.Pro325= (splicing defect), p.Arg296Cys, p.Ser486Thr	Yes	0-0.7%
Hybrid genes	no function	variable								

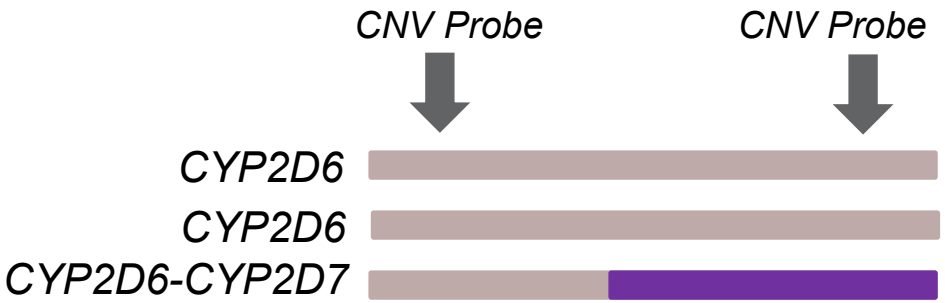
Do not meet MAF for Tier 1

Do not have reference materials; *12 and *49 may be promoted to Tier 1 if reference materials become available in the future

Meets standard criteria for Tier 1, but is defined by an in-frame insertion of 18 base pairs that is difficult to detect; therefore, assigned to Tier 2 based on technical difficulty

Tier 2 – Hybrid Alleles

- When one probe is used, differentiating between a duplication and hybrid is not possible; however, when 2 probes are used, this is feasible



Allele	Hybrid type	5' UTR†	exon 1†	intron 2†	intron 5†	intron 6†	exon 9†
*4.013 (*4N)	2D6-2D7	yes	yes	yes	yes	yes	no
*13	2D7-2D6	no	no	yes/no	yes/no	yes/no	yes‡,‖
*36	2D6-2D7	yes	yes	yes	yes	yes	no
*68	2D6-2D7	yes	yes	no‡	no‡	no‡	no
*83	2D6-2D7	yes	yes	yes	yes	yes	no

However, for a CYP2D6-CYP2D7 hybrid (which is more common than a *13 CYP2D7-CYP2D6 hybrid), this may not be a problem if the probe is located in exon 9

CYP2D6 Detection rate

- Tier 1
  - ≥78% of African-American
  - ~84% of European Caucasian
  - ~85% of East Asian
- Tier 2
  - ≥80% African-American
  - ~85% European Caucasian
  - ~87% East Asian

Non wild-type variant only in calculation (does not include all possibly duplications)

Tier 1

- *2, *3, *4, *5, *6, *9, *10, *17, *29, *41, duplications

Tier 2

- *7, *8, *12, *14, *15, *21, *31, *40, *42, *49, *56, *59, hybrids

AMP PGx Working Group *TPMT/NUDT15*

- 5th deliverable

The Journal of Molecular Diagnostics
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Special article

TPMT and *NUDT15* Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase

Victoria M. Pratt *, †, ✉, Larisa H. Cavallari *, ‡, Makenzie L. Fulmer *, §, Andrea Gaedigk *, ¶, Houda Hachad *, ‖, Yuan Ji *, §, Lisa V. Kalman *, **, Reynold C. Ly *, †, Ann M. Moyer *, ††, Stuart A. Scott *, ‡‡, §§, R.H.N. van Schaik *, ¶¶, ‖‖, ***, Michelle Whirl-Carrillo *, †††, Karen E. Weck *, ‡‡‡, §§§

AMP Guidelines for *TPMT/NUDT15* clinical testing – Tier 1

Allele	Allele Functional Status†	Core Variant(s)‖	RefSeqGene LRG Nomenclature	HGVS Genomic Nomenclature (GRCh38)	HGVS cDNA Nomenclature	HGVS protein Nomenclature	Reference Material Available	Multiethnic Allele Frequency (%)
*TPMT*2*	No Function	rs1800462	NG_012137.3: g.16420G>C	NC_000006.12: g.18143724C>G	NM_000367.5: c.238G>C	NP_000358.1: p.Ala80Pro	Yes‡	0-0.7
*TPMT*3A*	No Function	rs1800460, rs1142345	NG_012137.3: g.21147G>A, NG_012137.3: g.29457A>G	NC_000006.12: g.18138997C>T, NC_000006.12: g.18130687T>C	NM_000367.5: c.460G>A, NM_000367.5: c.719A>G	NP_000358.1: p.Ala154Thr, NP_000358.1: p.Tyr240Cys	Yes	0.03-4.2
*TPMT*3B*	No Function	rs1800460	NG_012137.3: g.21147G>A	NC_000006.12: g.18138997C>T	NM_000367.5: c.460G>A	NP_000358.1: p.Ala154Thr	Yes (*3A)	0-0.5
*TPMT*3C*	No Function	rs1142345	NG_012137.3: g.29457A>G	NC_000006.12: g.18130687T>C	NM_000367.5: c.719A>G	NP_000358.1: p.Tyr240Cys	Yes	0.6-5.3
*NUDT15*3*	No Function	rs116855232	NG_047021.1: g.13153C>T	NC_000013.11: g.48045719C>T	NM_018283.4: c.415C>T	NP_060753.1: p.Arg139Cys	Yes‡	0-6.8

AMP Guidelines for *TPMT/NUDT15* clinical testing – Tier 2

Allele	Allele Functional Status†	Core Variant(s)‖	RefSeqGene LRG Nomenclature	HGVS Genomic Nomenclature (GRCh38)	HGVS cDNA Nomenclature	HGVS protein Nomenclature	Reference Material Available	Multiethnic Allele Frequency (%)
*TPMT*11*	No Function	rs72552738	NG_012137.3: g.20455G>A	NC_000006.12: g.18139689C>T	NM_000367.5: c.395G>A	NP_000358.1: p.Cys132Tyr	No	0-0.02
*TPMT*29*	No Function	rs267607275	NG_012137.3: g.11018T>C	NC_000006.12: g.18149126A>G	NM_000367.5: c.2T>C	NP_000358.1: p.Met1Thr	No	0-0.03
*TPMT*42*	Likely No Function	rs759836180	NG_012137.3: g.11111dup	NC_000006.12: g.18149034dup4	NM_000367.5: c.95dup	NP_000358.1: p.Trp33Valfs*26	No	0-0.11
*NUDT15*2*	No Function	rs746071566, rs116855232	NG_047021.1: g.5218GAGTCG[4], NG_047021.1: g.13153C>T	NC_000013.11: g.48037796_48037801dup, NC_000013.11: g.48045719C>T	NM_018283.4: c.50_55dup, NM_018283.4: c.415C>T	NP_060753.1: p.Gly17_Val18dup, NP_060753.1: p.Arg139Cys	Yes‡	0-3.7
*NUDT15*4*	Uncertain Function	rs147390019	NG_047021.1: g.13154G>A	NC_000013.11: g.48045720G>A	NM_018283.4: c.416G>A	NP_060753.1: p.Arg139His	Yes	0-1.8
*NUDT15*6*	Uncertain Function	rs746071566	NG_047021.1: g.5218GAGTCG[4]	NC_000013.11: g.48037796_48037801dup	NM_018283.4: c.50_55dup	NP_060753.1: p.Gly17_Val18dup	Yes‡	0-1.3
*NUDT15*9*	No Function	rs746071566	NG_047021.1: g.5218GAGTCG[2]	NC_000013.11: g.48037796_48037801del	NM_018283.4: c.50_55del	NP_060753.1: p.Gly17_Val18del	Yes‡	0-0.2
*NUDT15*14*	Likely No Function	rs777311140	NG_047021.1: g.5260_5261insCGGG	NC_000013.11: g.48037826_48037827insCGGG	NM_018283.4: c.80_81insCGGG	NP_060753.1: p.Cys28Glyfs*28	No	0-0.6

AMP PGx Working Group Genotyping Recommendations

These recommendations are intended to:

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

Future work:

- Other PGx genes with clinical relevance planned/ in progress

Testing (r)evolution

Targeted

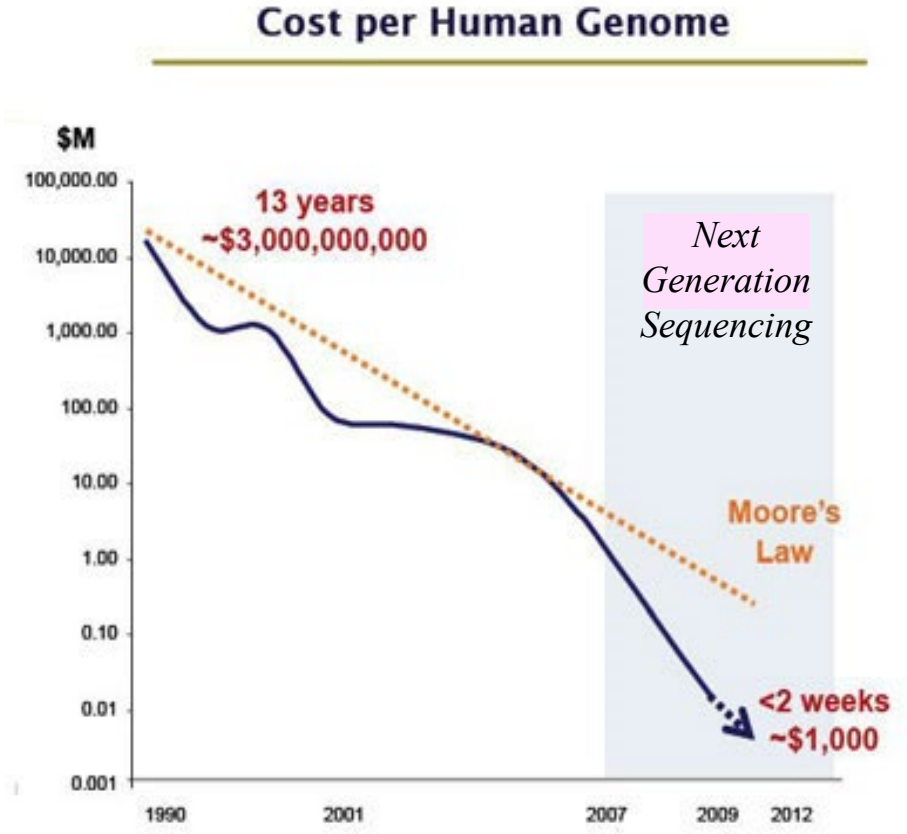
Whole “omic” approach

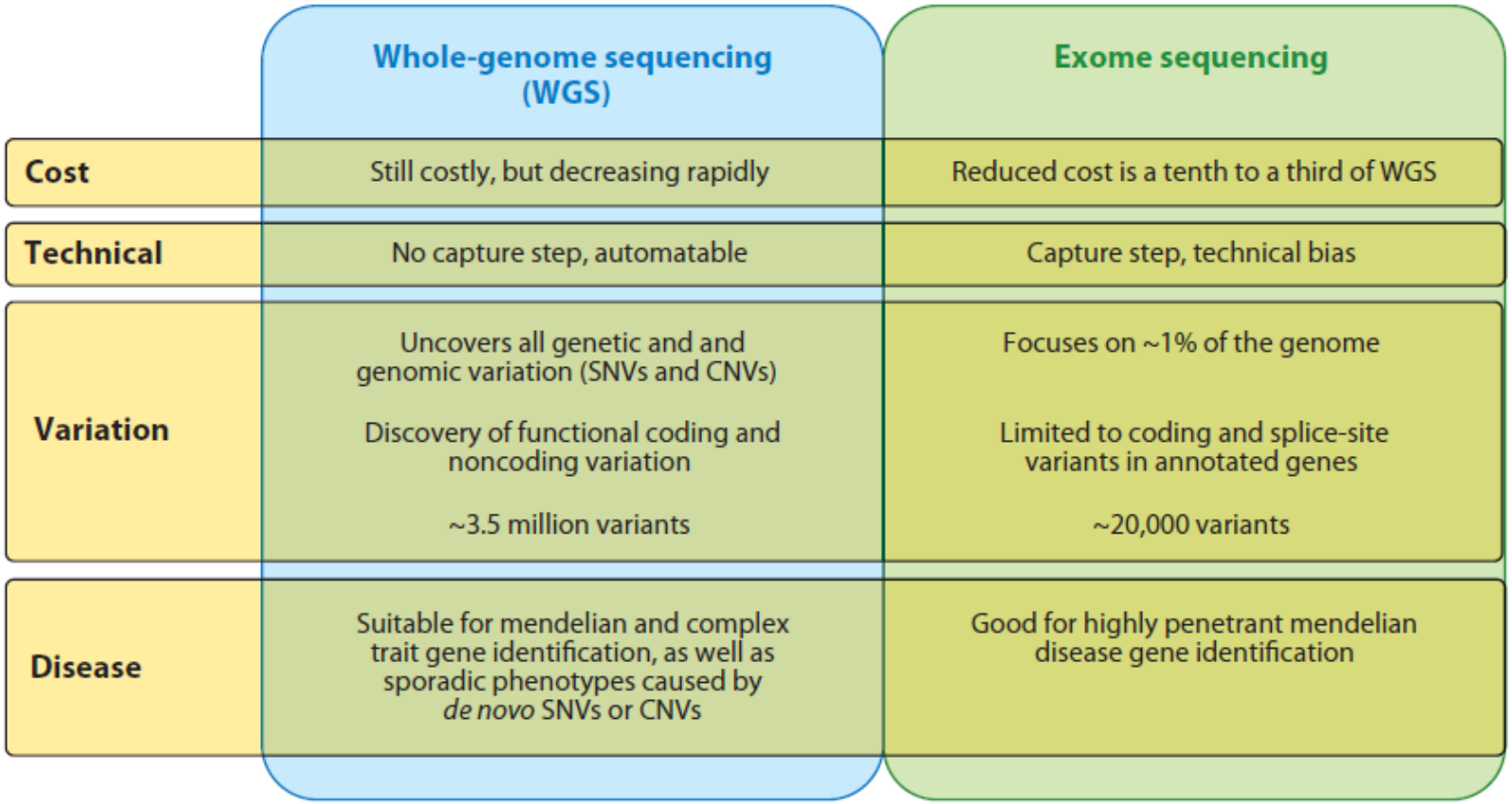
Risk Icon On Speedometer High Risk Meter Vector Stock Illustration Stock Illustration - Download Image Now - iStock (istockphoto.com)
https://www.alamy.com/stock-photo-young-strong-woman-warrior-with-big-guns-in-dramatic-urban-night-scene-145283794.html

WHOLE “OMIC” APPROACH

https://www.alamy.com/stock-photo-young-strong-woman-warrior-with-big-guns-in-dramatic-urban-night-scene-145283794.html

Accelerating Technology & Plummeting Cost





	Whole-genome sequencing (WGS)	Exome sequencing
Cost	Still costly, but decreasing rapidly	Reduced cost is a tenth to a third of WGS
Technical	No capture step, automatable	Capture step, technical bias
Variation	Uncovers all genetic and and genomic variation (SNVs and CNVs) Discovery of functional coding and noncoding variation ~3.5 million variants	Focuses on ~1% of the genome Limited to coding and splice-site variants in annotated genes ~20,000 variants
Disease	Suitable for mendelian and complex trait gene identification, as well as sporadic phenotypes caused by *de novo* SNVs or CNVs	Good for highly penetrant mendelian disease gene identification

Figure 3

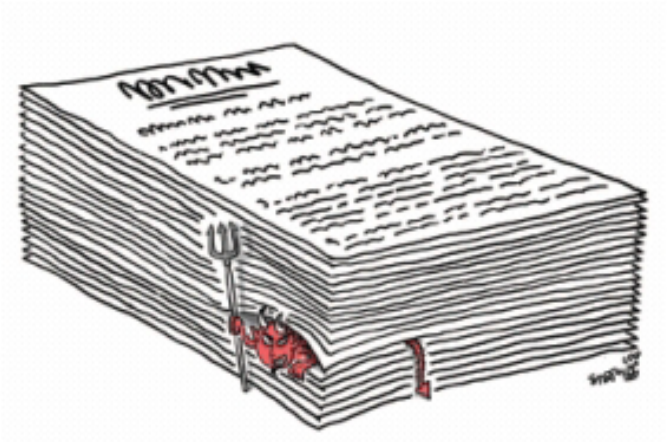
A comparison of the weaknesses and strengths of whole-genome sequencing (WGS) and exome sequencing approaches for disease-gene identification. Abbreviations: CNVs, copy-number variants; SNVs, simple nucleotide variants.

Gonzaga-Jauregui, Lupski and Gibbs, Ann Rev Med 63: 35-61, 2012.

The Devil is in the Details

NGS (short read) HAS PROBLEMS WITH

- Regions of high sequence homology
- Repeat expansions
- Large in/dels, CNVs and other structural variants



http://www.trizic.com/the-devil-is-in-the-details-how-prepared-are-you-for-a-presence-exam/

LESS PROBLEMATIC FOR GENE PANELS

- Testing labs typically have clinical domain expertise
- Contents typically highly curated + well understood
- Add-on tests typically available for difficult genes

KNOWLEDGE DOES NOT SCALE EASILY

- Sequencing ≠ understanding

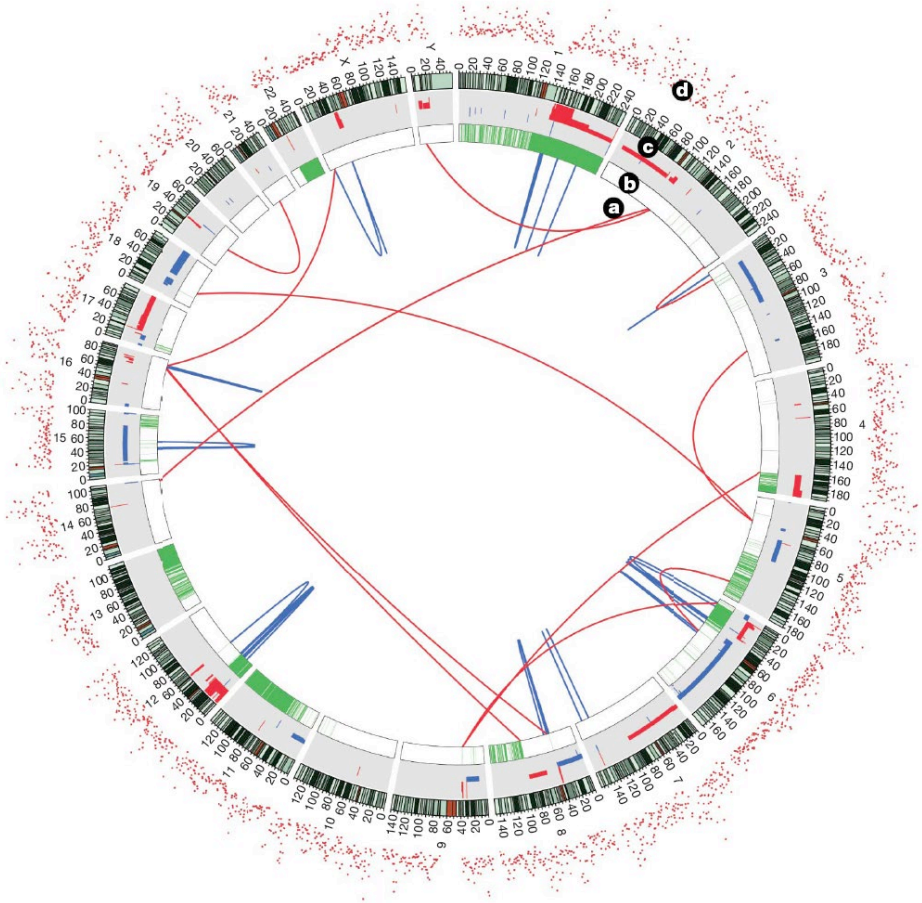
Courtesy: Birgit Funke

HOW MANY HOURS DO MOLECULAR PROFESSIONALS SPEND ANALYZING, INTERPRETING, AND REPORTING MOLECULAR TESTS FOR ONCOLOGY?

https://www.amp.org/AMP/assets/File/advocacy/AMP_MDx_Interpretation_Quant_Survey_Report.pdf?pass=86

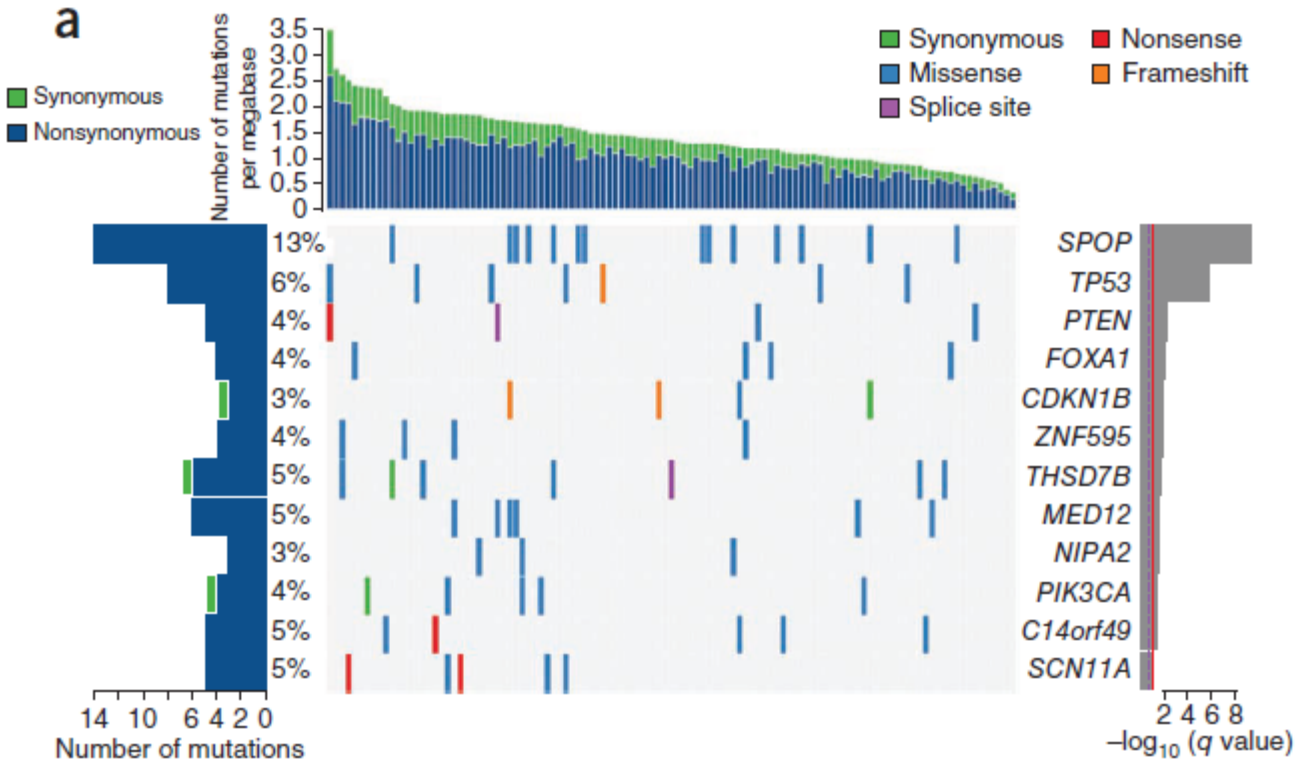
CANCER

Cancer: Tumor vs. Normal



Lee et al. Nature 2010

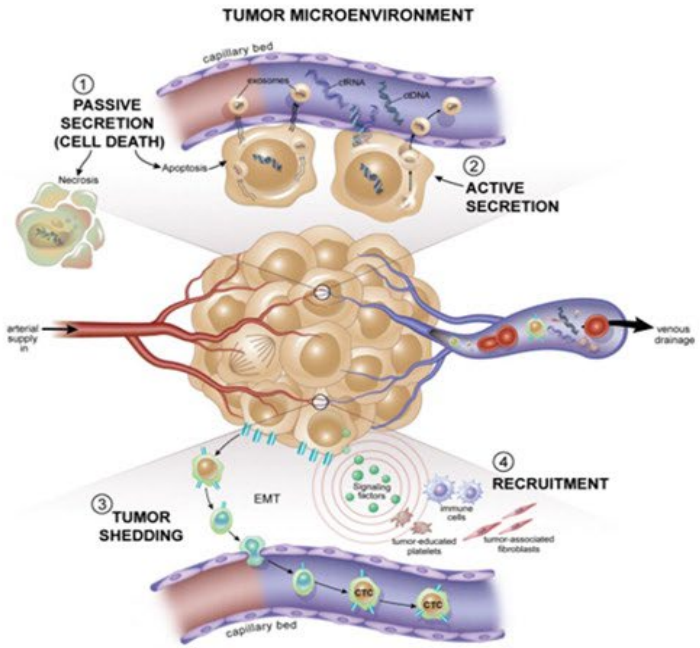
Exome Sequencing in Prostate Cancer



Barbieri et al. Nature Genetics 2012

Liquid biopsy

Liquid Biopsy for Cancer: Review and Implications

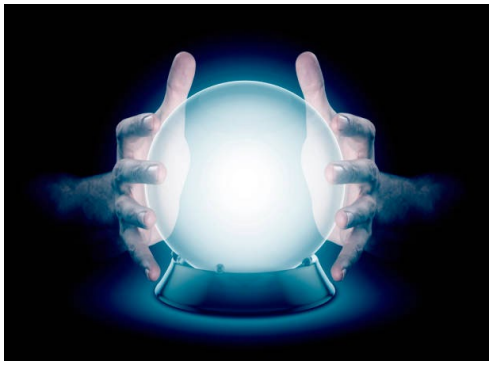


Mechanisms of translocation of tumor cells and cellular components into the bloodstream

- Liquid biopsy is a noninvasive diagnostic approach involving the isolation of circulating tumor markers such as cell-free nucleic acids and circulating tumor cells from peripheral blood
- The tumor microenvironment hosts growing and apoptotic cancer cells that release biomarkers into the circulation, which can be collected for the purpose of analyzing tumor biology
- Circulating biomarkers including circulating tumor DNA and circulating tumor cells can serve as noninvasive tests for screening, diagnosis, prognosis, and therapy guidance for many solid tumors. Methods are being developed to detect and characterize these markers

Underwood JJ et al. Published Online: November 19, 2019
https://doi.org/10.1148/radiol.2019182584

Radiology



Future

- Polygenic risk score tests
- Whole genome + Whole transcriptome
- Methylome
- ???

https://pixels.com/featured/9-hands-on-crystal-ball-and-cryptocurrency-allan-swart.html

Is more better?

- My thoughts….

  Sometimes yes

  Sometimes no

Conclusions

- Genetics is continually evolving field
- Some resources are available to help with understanding level of evidence for genes in panels

  Our understanding is constantly evolving

Questions