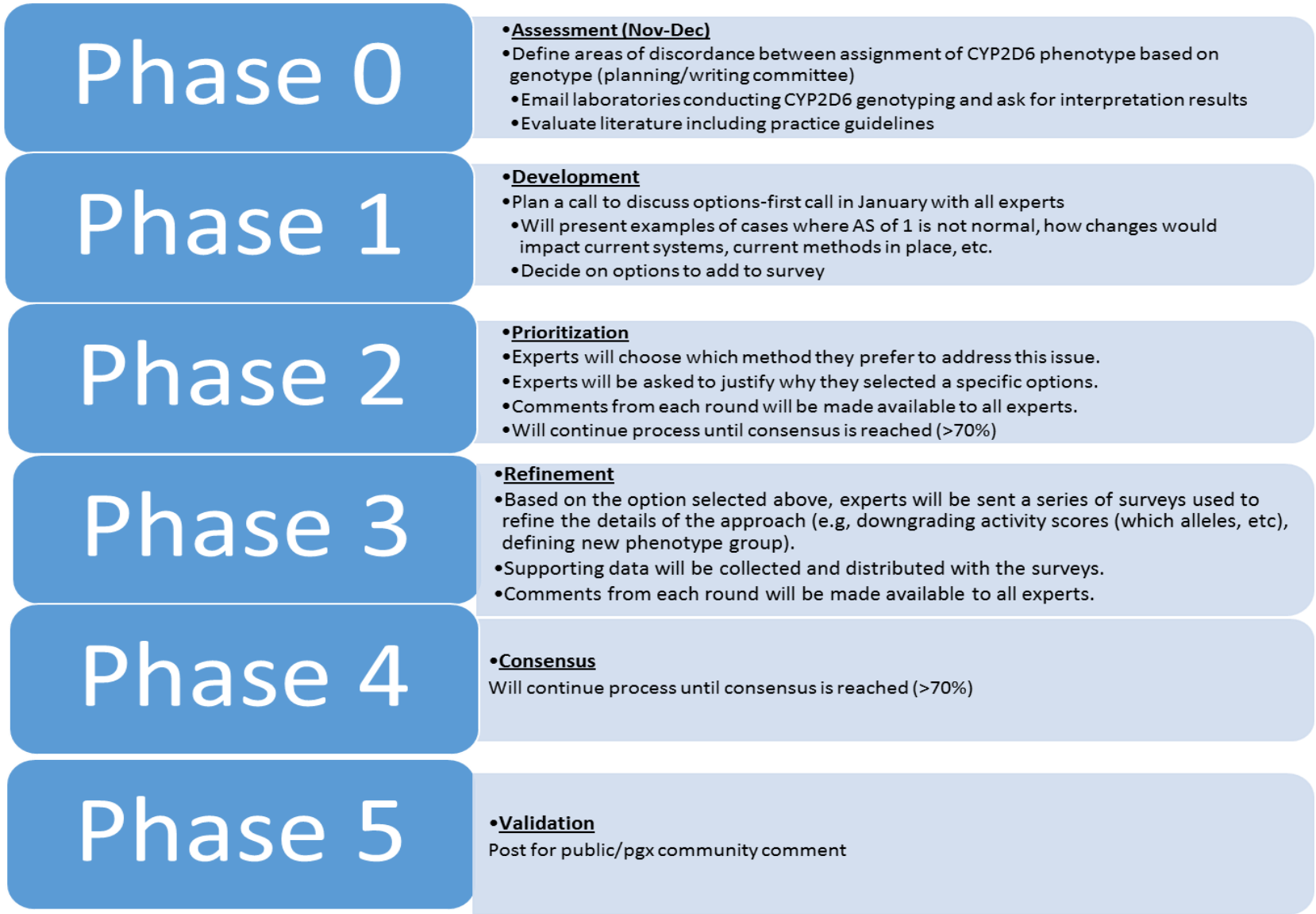
CYP2D6 Genotype to Phenotype Standardization Project

Project Objectives

- Determine a strategy for defining CYP2D6 phenotype based on genotype using a modified Delphi method.

- Standardize phenotype definitions across CPIC and DPWG guideline and external groups.

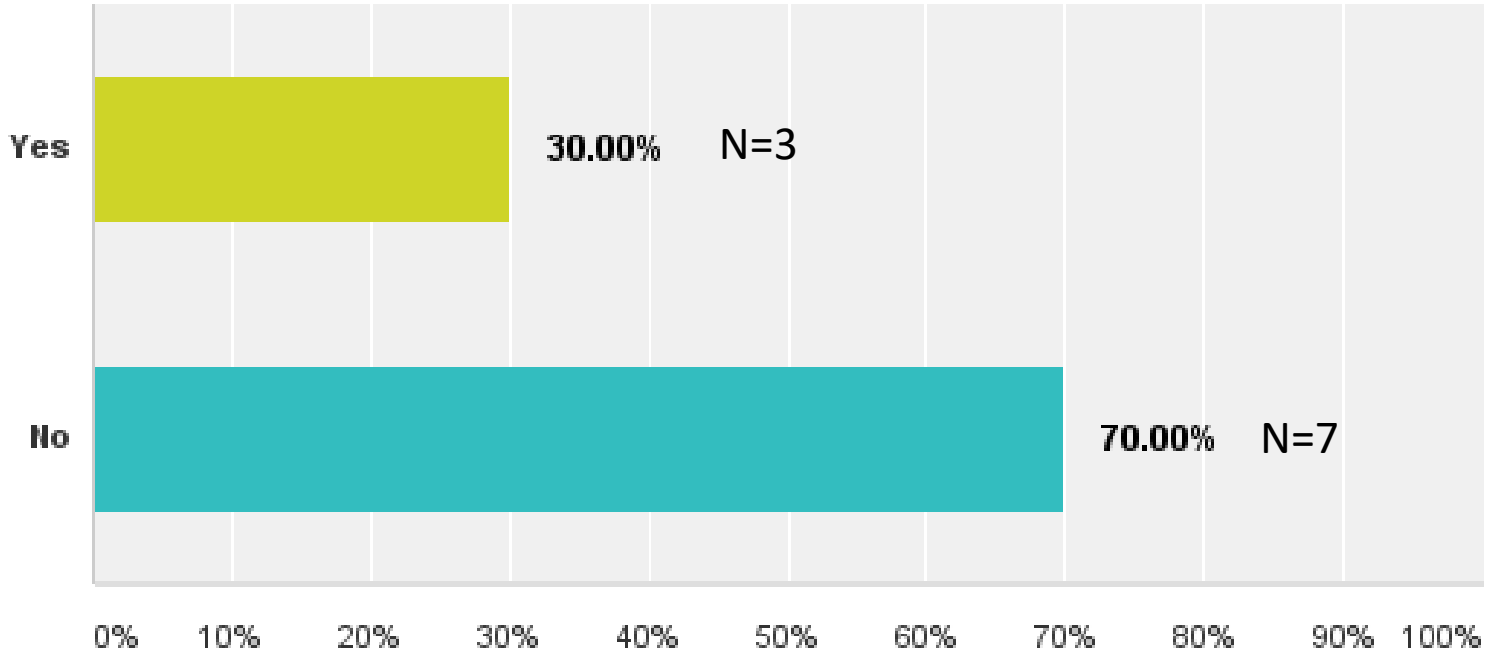

Phase 0
•Assessment (Nov-Dec)
•Define areas of discordance between assignment of CYP2D6 phenotype based on genotype (planning/writing committee)
•Email laboratories conducting CYP2D6 genotyping and ask for interpretation results
•Evaluate literature including practice guidelines
Phase 1
•Development
•Plan a call to discuss options-first call in January with all experts
•Will present examples of cases where AS of 1 is not normal, how changes would impact current systems, current methods in place, etc.
•Decide on options to add to survey
Phase 2
•Prioritization
•Experts will choose which method they prefer to address this issue.
•Experts will be asked to justify why they selected a specific options.
•Comments from each round will be made available to all experts.
•Will continue process until consensus is reached (>70%)
Phase 3
•Refinement
•Based on the option selected above, experts will be sent a series of surveys used to refine the details of the approach (e.g, downgrading activity scores (which alleles, etc), defining new phenotype group).
•Supporting data will be collected and distributed with the surveys.
•Comments from each round will be made available to all experts.
Phase 4
•Consensus
Will continue process until consensus is reached (>70%)
Phase 5
•Validation
Post for public/pgx community comment

Phase 0: Survey to genetic testing laboratories

- Sent survey to 43 genetic testing laboratories (CPIC members and labs listed in the GTR)
- 23 started survey; 10 labs completed survey

Q7 Do you use the activity score as described in CPIC guidelines (Gaedigk, et al., 2008, Crews et al., 2014), to translate CYP2D6 genotype into phenotype?

Answered: 10 Skipped: 0



CPIC

Table 1 Assignment of likely codeine metabolism phenotypes based on cytochrome P450 2D6 (*CYP2D6*) diplotypes

Likely phenotype[a]	Activity score	Genotypes	Examples of diplotypes
Ultrarapid metabolizer (~1–2% of patients)	>2.0	An individual carrying more than two copies of functional alleles	**1/*1xN, *1/*2xN*
Normal metabolizer (~77–92% of patients)	1.0–2.0[b]	An individual carrying two alleles encoding full or reduced function; or one full-function allele together with either one nonfunctional or one reduced-function allele	**1/*1, *1/*2, *2/*2, *1/*41, *1/*4, *2/*5, *1/*10*
Intermediate metabolizer (~2–11% of patients)	0.5[b]	An individual carrying one reduced-function and one nonfunctional allele	**4/*10, *5/*41*
Poor metabolizer (~5–10% of patients)	0	An individual carrying no functional alleles	**4/*4, *4/*5, *5/*5, *4/*6*

[a]The frequency estimates are based on data from Caucasians and may differ substantially for other ethnicities. See **Supplementary Data** online for estimates of phenotype frequencies among different ethnic/geographic groups. [b]Note that some investigators define patients with an activity score of 0.5 and 1.0 as intermediate metabolizers and those with an activity score of 1.5 and 2.0 as extensive metabolizers. Classifying patients with an activity score of 1.0 as extensive metabolizers in this guideline is based on data specific for formation of morphine from codeine in these patients.[12]

DPWG

Phenotype (Genotype)
PM (two inactive (*3-*8, *11-*16, *19-*21, *38, *40, *42) alleles)
IM (two decreased-activity (*9, *10, *17, *29, *36, *41) alleles or carrying one active (*1, *2, *33, *35) and one inactive (*3-*8, *11-*16, *19-*21, *38, *40, *42) allele, or carrying one decreased-activity (*9, *10, *17, *29, *36, *41) allele and one inactive (*3-*8, *11-*16, *19-*21, *38, *40, *42) allele)
UM (a gene duplication in absence of inactive (*3-*8, *11-*16, *19-*21, *38, *40, *42) or decreased-activity (*9, *10, *17, *29, *36, *41) alleles)

Comparison

Phenotype	CPIC	DPWG	Lab 1	Lab 2	Lab 3	Lab 4	Luminex	
Ultrarapid Metabolizer	>2	>2		3	>2	>2		
NM to UM				2.5				
Normal Metabolizer	1 to 2	1.5-2		2	2	2	1 to 2	(where 1 is the combination of 1 normal and 1 no/decreaed function allele)
IM to NM				1.5				
Intermediate Metabolizer	0.5	0.5-1	0.75≤x≤1.25	1	0.5-1	0.5-1	0.5 to 1	(where 1 is the combination of 2 decreased function alleles)
PM to IM			0<x<0.75	0.5				
Poor Metabolizer	0	0	0	0	0	0		

Questions

- Are there differences between an AS of 1 and 2?
- Are there differences between an AS of 0.5 and 1?
- Are there alleles which should receive a lower value than 0.5 for AS calculation?

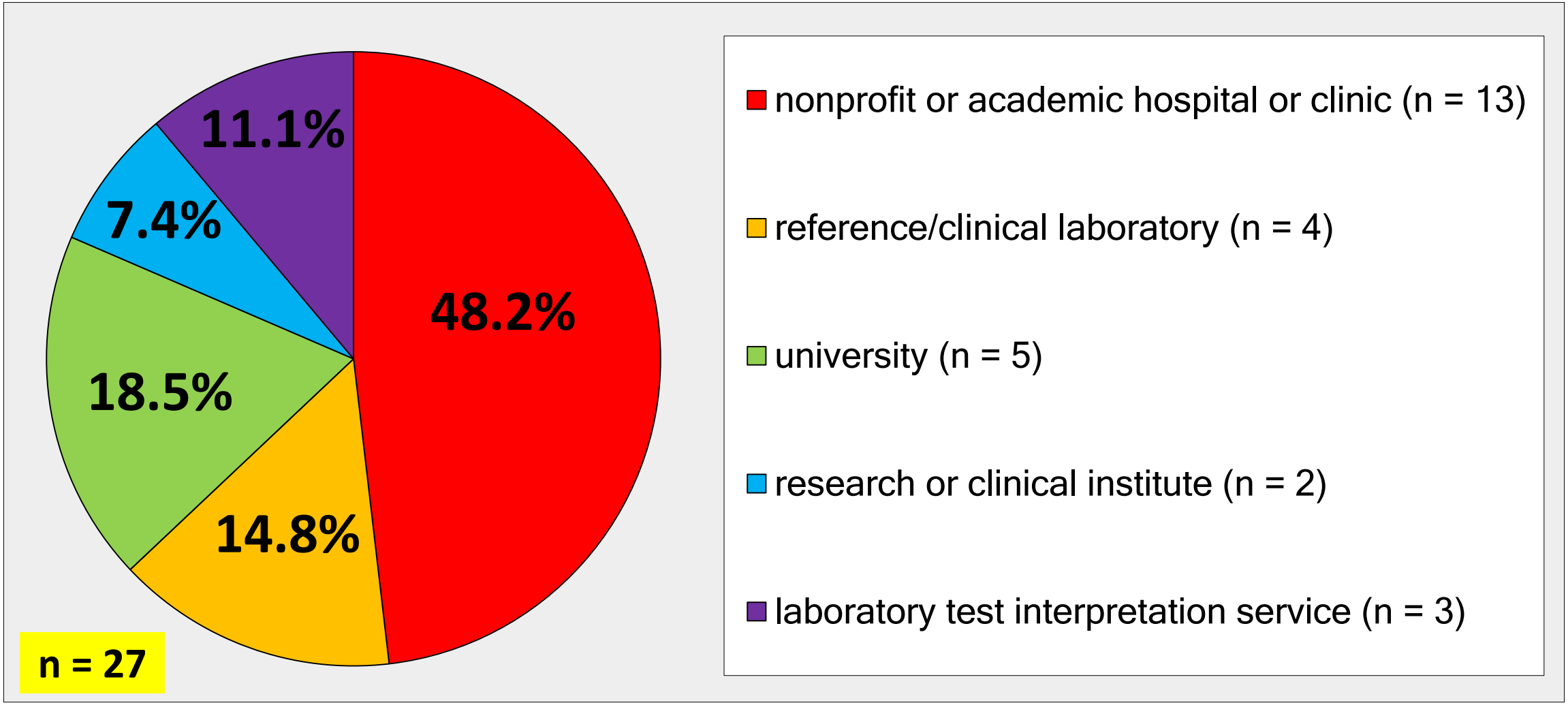
Summary

Drug	AS 1 vs 2	AS 0.5 vs 1	Comments
Atomoxetine	yes	yes	Data strongest for AS 1 vs 2 in *10 containing diplotypes
Paroxetine	yes	yes	small numbers
Venlafaxine	yes	yes	
Risperidone	yes	yes	
Aripiprazole	yes	yes	
Nortriptyline	yes	not compared	
Dextromethorphan	yes	yes	Data strongest for AS 1 vs 2 in *10 containing diplotypes
Codiene	yes	not compared	Only data for AS 1 vs 2 in *10 containing diplotypes
Tamoxifen	yes	not compared	
Metoprolol	yes	not compared	

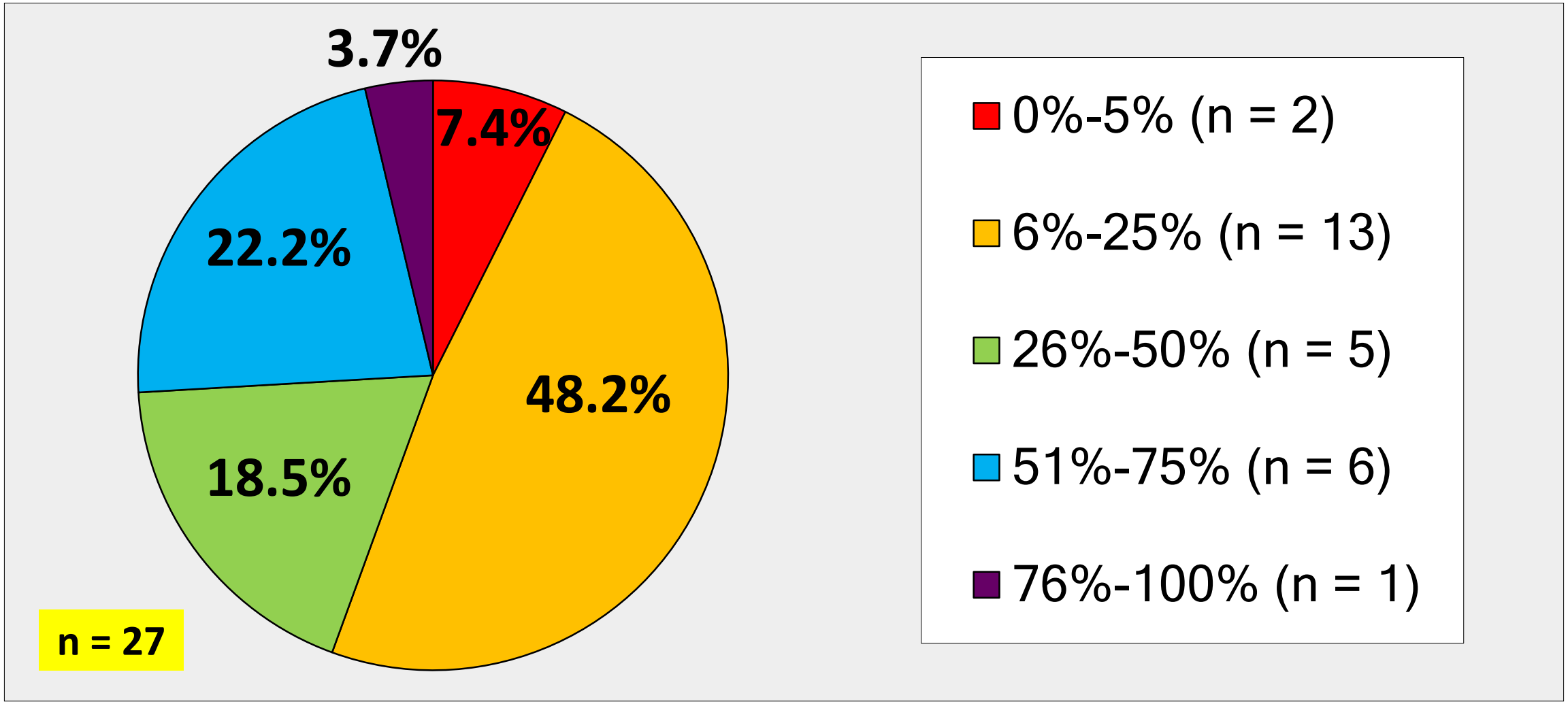
Possible Solutions

- Define a new allele function group, basically “severely decreased” or “low” function, with an activity score of 0.25.
  - Identify alleles falling into this category.
  - This would also create AS groups of 0.75, 1.25, and 2.25, which need to be categorized.
  - If going this route, genotype combination of a normal function and a no function allele could receive an IM assignment.
- Create a new metabolizer phenotype category for the CYP2D6 AS of 0.5 and assigning an AS of 1 as intermediate metabolizer phenotype.
  - Based on the current system individuals with a no and decreased function allele will be assigned this new phenotype category. It would also mean *10/*10 would still be grouped together with *9/*9 and *1/*4.
- Assign the IM category to an AS of 0.5 and 1.
  - Is it sufficient for the PGx community that a range of *5/*10 to *1/*4 is grouped together and gets the same recommendation.
- Use Gaedigk activity score, as is but have separate recommendations based on AS.
  - a NM will still be AS of 1-2 but the recommendation might be different based on AS.

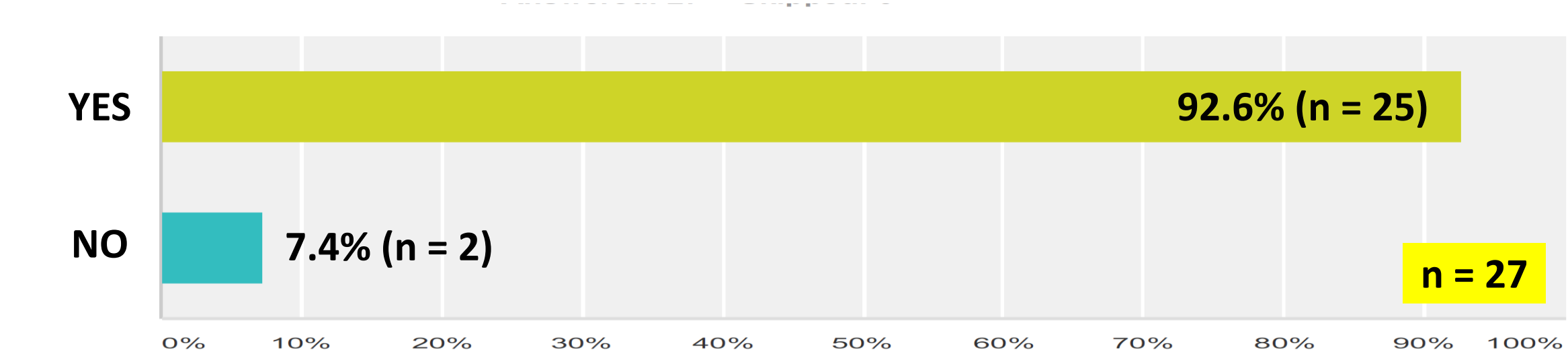
Demographics: Workplace setting



Demographics: % of work involving CYP2D6



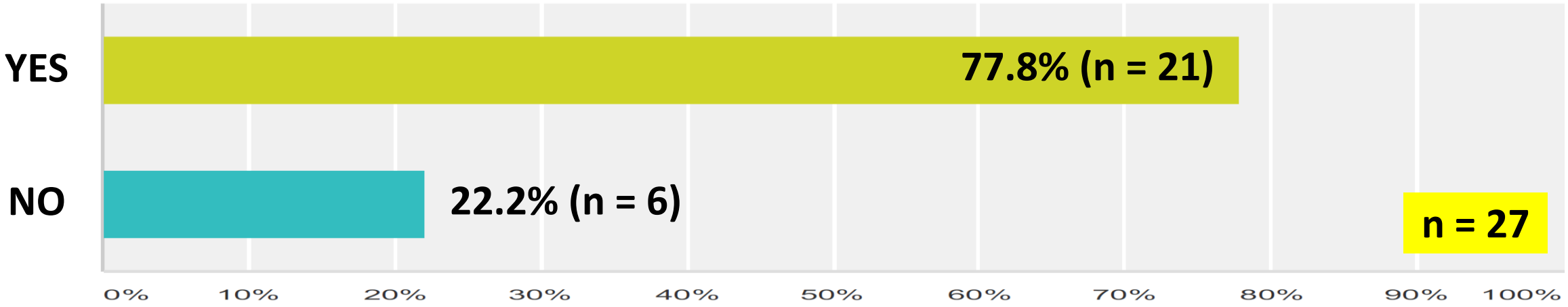
Q1: Clinically significant difference between AS 1 and 2?



Select comments:

- Substrate-specific
- Protein expression as well as functional activity is significantly reduced in AS 1 vs. AS 2
- Definitely PK differences
- PK differences may or may not equate to clinical differences (drug-specific – depends on therapeutic index)
- Strongest difference between *10/*10 and *1/*1 (difference may not exist if *10 is not included in analysis)
- Appears to be difference when *10 included in analysis, but not in studies that do not include *10
- AS 1 may be more prone to interactions with CYP2D6 inhibitor vs. AS 2
- If difference wasn’t found, it was due to small sample size

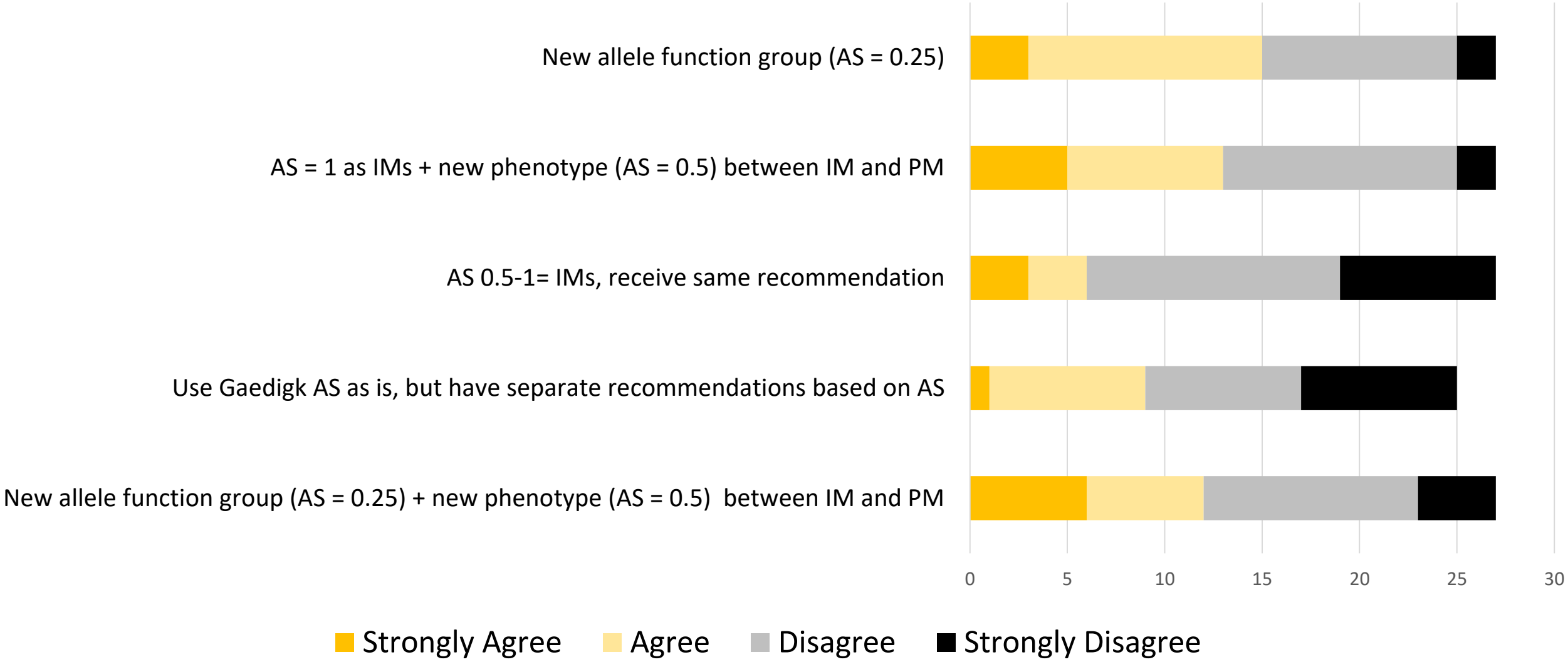
Q2: Clinically significant difference between AS 0.5 and 1?



Select comments:

- Substrate-specific
- Difficult to determine from available data – need more research
- Studies that show a difference have a larger sample size than studies that don't
- Less data to support difference
- May be statistical difference but likely no clinical difference
- Best to distinguish between the two to account for the cases where the difference may be relevant
- Recommend keeping phenotypes as detailed as possible to avoid discrepancies between studies that can complicate interpretation when various genotype combinations are pooled in same phenotype

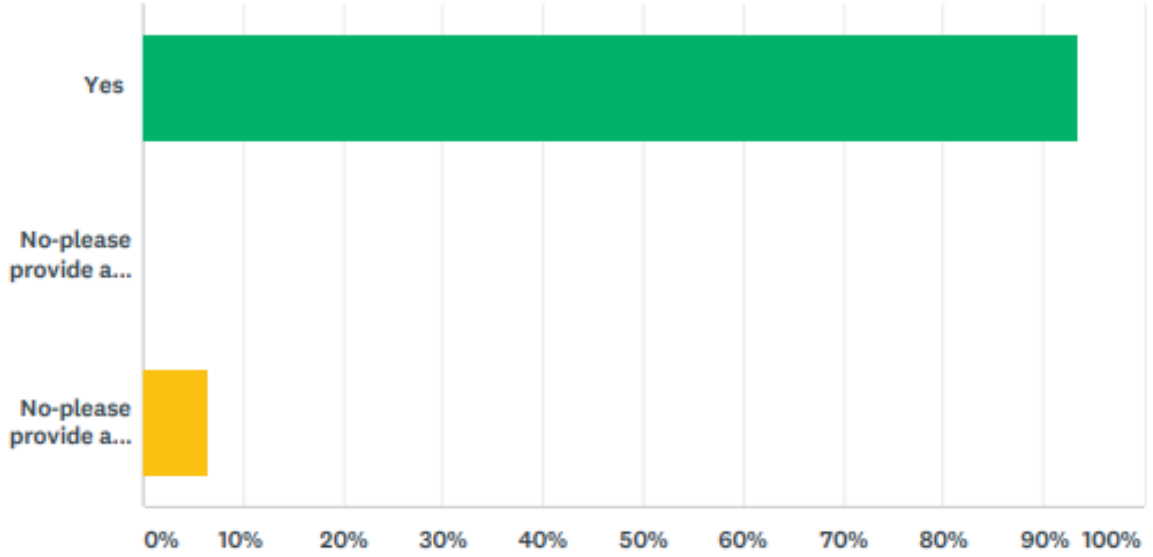
Q5: Which method to convert genotype to phenotype?



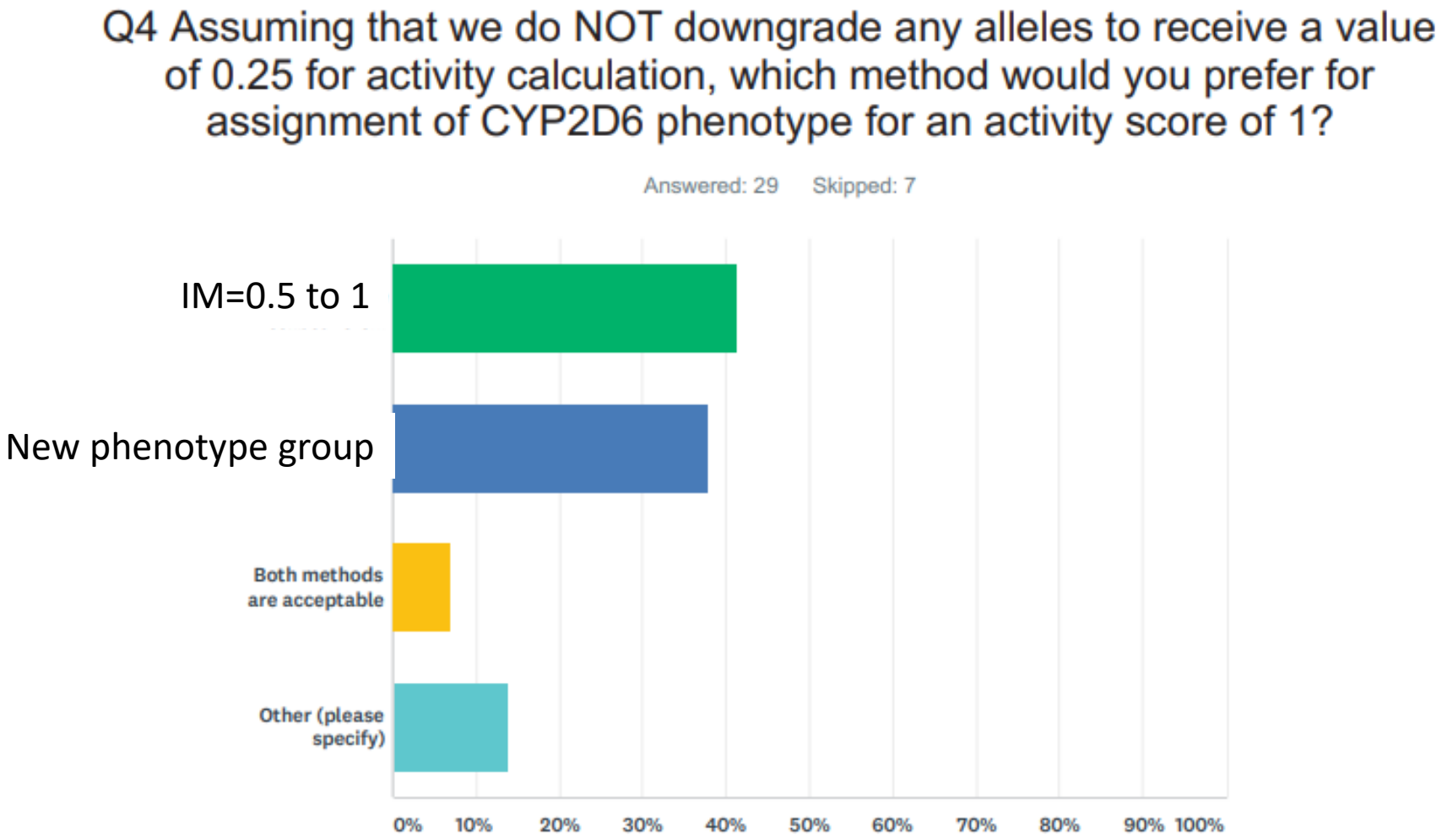
Survey 2 results

Q3 Do you agree with this recommendation to proceed with the use of the activity scores, the possibility of downgrading some alleles to a new functional group (AS = 0.25; severely decreased function-assuming we have convincing data) and categorizing AS = 1 as intermediate metabolizers AND/OR creating a new phenotype group between IM and PM?

Answered: 31 Skipped: 5



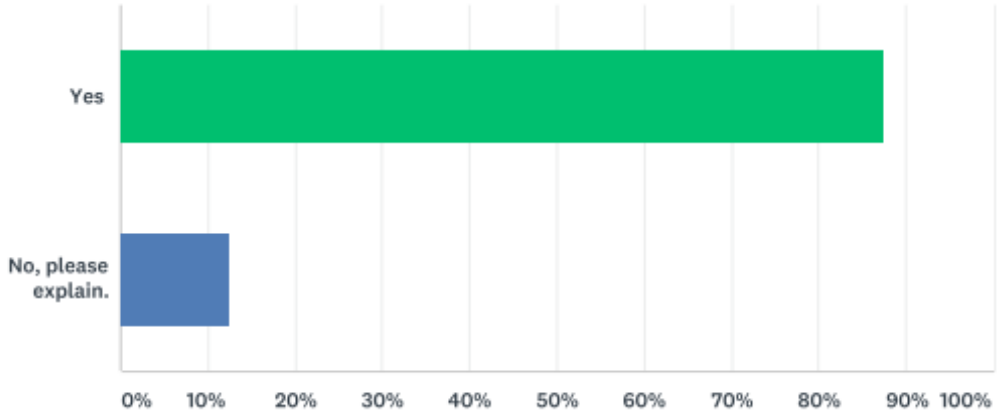
Survey 2 results



Survey 3 results

Q3 Do you agree with this recommendation to proceed with categorizing AS=1 as intermediate metabolizers (i.e. IM=0.5 to 1)? Please note: Additional details of assignment of phenotype based on genotype (e.g., how to incorporate AS of 2.5) will be decided in subsequent surveys.

Answered: 24 Skipped: 0

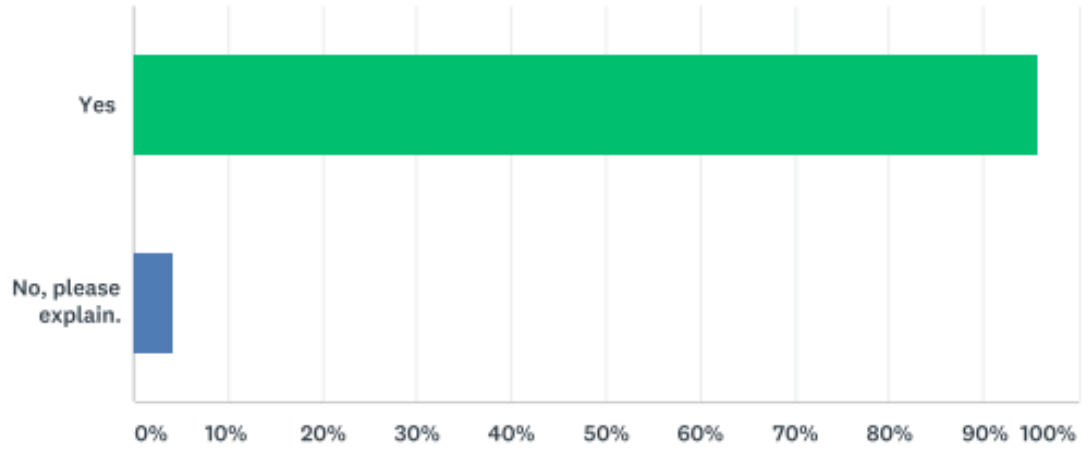


ANSWER CHOICES	RESPONSES	
Yes	87.50%	21
No, please explain.	12.50%	3
TOTAL		24

Survey 3 results

Q4 Do you agree with this recommendation to proceed with downgrading some alleles (*10 for now) to a lower AS score (0.25)? Please note: Additional details of assignment of phenotype based on genotype (e.g., how to incorporate AS of 0.25) will be decided in subsequent surveys.

Answered: 24 Skipped: 0

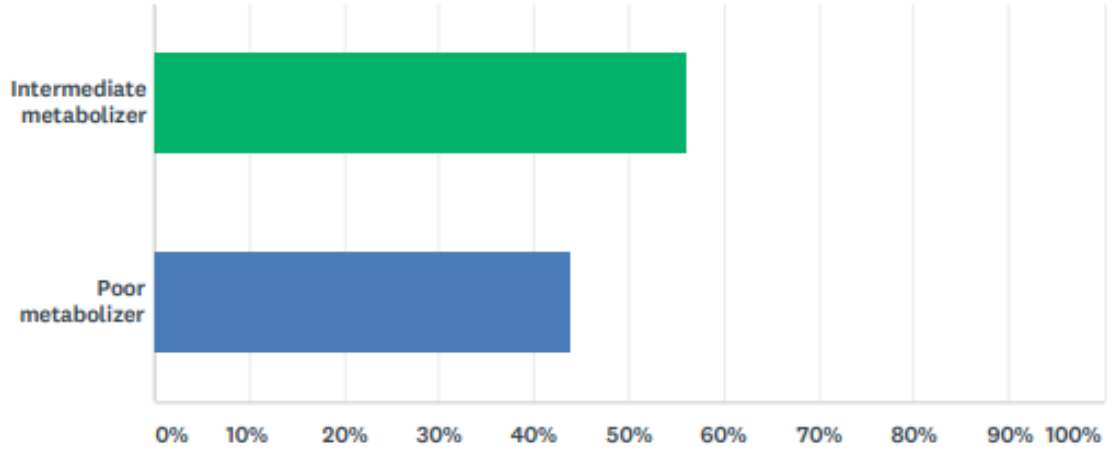


ANSWER CHOICES	RESPONSES	
Yes	95.83%	23
No, please explain.	4.17%	1
TOTAL		24

Survey 4 results

Q1 Do you think an AS of 0.25 should be grouped as an IM or PM? Examples of AS of 0.25 include: CYP2D6*4/*10; *5/*10

Answered: 25 Skipped: 0



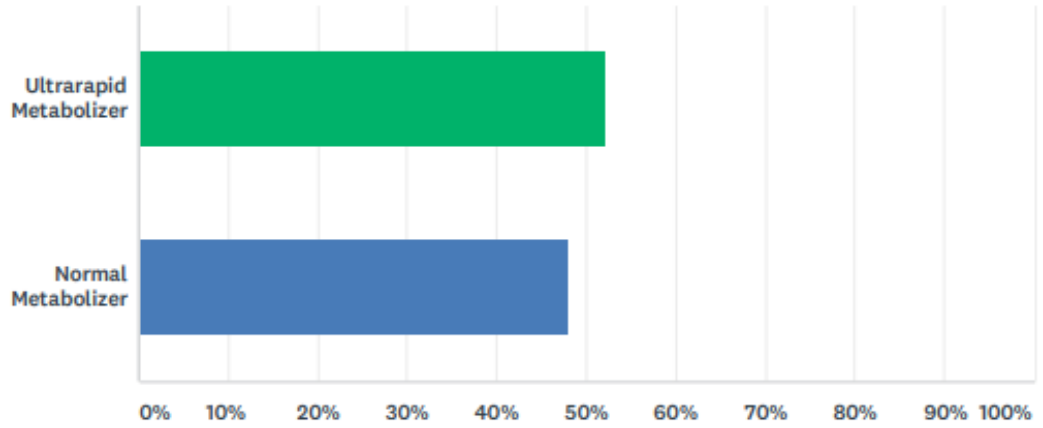
ANSWER CHOICES	RESPONSES	
Intermediate metabolizer	56.00%	14
Poor metabolizer	44.00%	11
TOTAL		25

Survey 4 results

CYP2D6 genotype to phenotype survey 4

Q2 Do you think an AS of 2.25 should be grouped as an UM or NM? Example includes: CYP2D6*1xN/*10.

Answered: 25 Skipped: 0

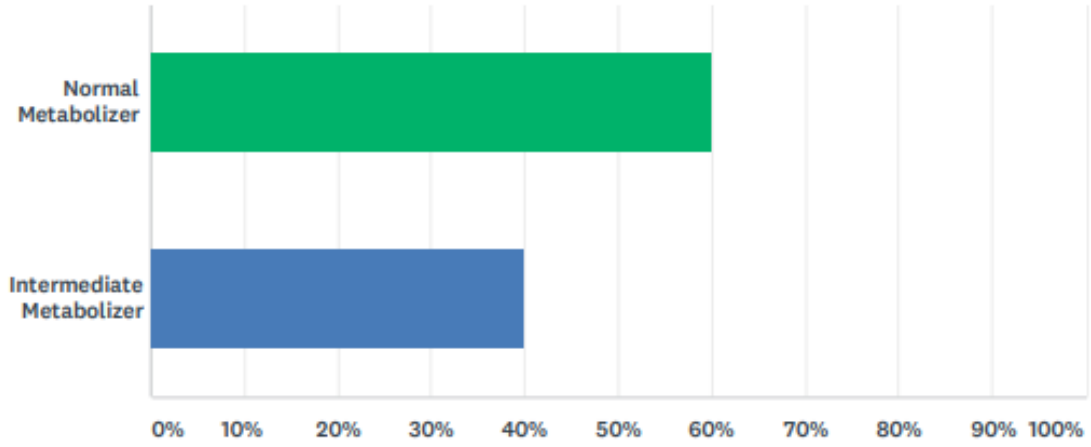


ANSWER CHOICES	RESPONSES	
Ultrarapid Metabolizer	52.00%	13
Normal Metabolizer	48.00%	12
TOTAL		25

Survey 4 results

Q3 Do you think an AS of 1.25 should be grouped as a NM or IM? Examples include: CYP2D6 *1/*10; *2/*10.

Answered: 25 Skipped: 0



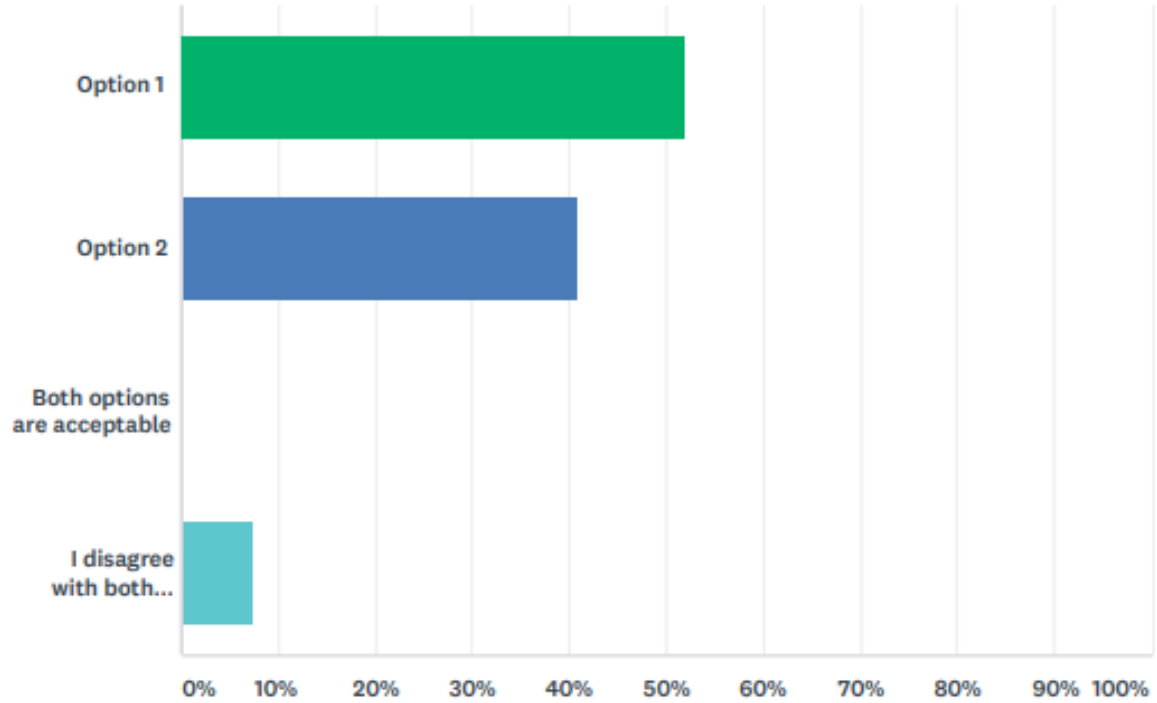
ANSWER CHOICES	RESPONSES	
Normal Metabolizer	60.00%	15
Intermediate Metabolizer	40.00%	10
TOTAL		25

Survey 5

	Option 1	Option 2
Ultrarapid Metabolizer	>2.25	>2
Normal Metabolizer	1.25 – 2.25	1.25-2
Intermediate Metabolizer	0.25-1	0.25-1
Poor Metabolizer	0	0

Q1 Which option do you prefer?

Answered: 27 Skipped: 0

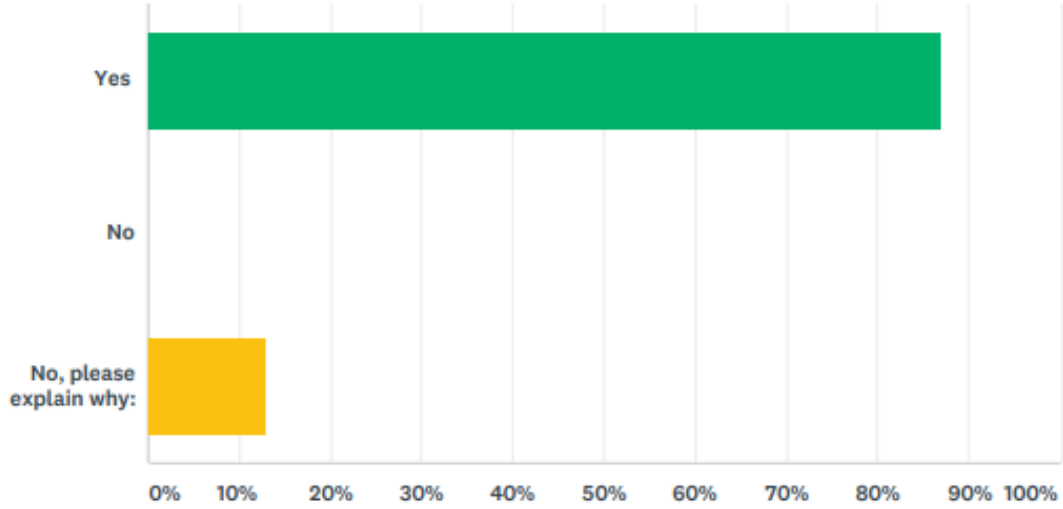


ANSWER CHOICES	RESPONSES	
Option 1	51.85%	14
Option 2	40.74%	11
Both options are acceptable	0.00%	0
I disagree with both options	7.41%	2
TOTAL		27

Survey 6 results

Q1 Do you agree with this recommendation to proceed with option 1?

Answered: 31 Skipped: 0



ANSWER CHOICES	RESPONSES	
Yes	87.10%	27
No	0.00%	0
No, please explain why:	12.90%	4
TOTAL		31

After 6 surveys, we have consensus!!

CYP2D6 Genotype to Phenotype table (current vs new)				
Likely phenotype	CURRENT CPIC activity score definition	CURRENT DPWG activity score definition	NEW standardized activity score definition	Examples of CYP2D6 diplotypes for new system
CYP2D6 ultrarapid metabolizer	>2	>2.5	> 2.25	*1/*1xN, *1/*2xN, *2/*2xN, *2x2/*9
CYP2D6 normal metabolizer	1-2	1.5-2.5	1.25-2.25	*1/*1, *1/*2, *1/*9, *1/*41, *2/*2, *1/*10, *2x2/*10
CYP2D6 intermediate metabolizer	0.5	0.5-1	0.25-1	*4/*10, *4/*41, *1/*5, *10/*10, *41/*41
CYP2D6 poor metabolizer	0	0	0	*3/*4, *4/*4, *5/*5, *5/*6

Rationale of downgrading an AS of 1 to the IM group:

- Experts were more in favor of this option albeit very close (41% vs 38%).
  - The option of classifying AS=1 as IM appears to be more likely to be accepted across all interest groups compared to a method that creates a new phenotype group for AS=0.5.
- After a consensus is reached, the recommendation for clinical labs would be to utilize this standardized classification.
  - Based on our survey results, laboratory experts were more in favor of classifying AS of 0.5 to 1 as CYP2D6 IMs than creating a new phenotype group.
  - More reporting labs currently classify AS of 0.5 to 1 as CYP2D6 IMs (**Table 2**).
- Recommendations from CPIC could be different for AS=0.5 and 1 if needed.
- Published studies vary on how they group activity scores for comparison. Some studies compare AS of 0.5-1 vs 2 while others compare AS of 1 vs 2. Classifying an AS of 1 as IM can be viewed as a more conservative approach guiding therapy, however, this grouping may not reveal potentially important differences among AS of 0.5 and 1.

Rationale for downgrading *CYP2D6*10* from 0.5 to 0.25:

- *CYP2D6*10* has been characterized as an allele conveying decreased function for a number of substrates. Although its activity ranges, it appears to be, in average, considerably lower compared to other decreased function alleles.
- The activity for subjects with CYP2D6*10/*10 (AS=1) or *10/no function (AS=0.5) diplotypes may therefore be over-estimated even when an AS of 1 is classified as IM.
- Assigning a value of 0.25 to the CYP2D6*10 allele for AS calculation will group *10/*10 as AS=0.5 and *10/no function as AS=0.25; the former will still be classified as IM, but would be in a group for which CPIC may identify a special recommendation. The introduction of a value of 0.25 creates the option of grouping subjects with an AS=0.25 with severely reduced activity as PMs.

Rational for AS of 2.25 assignment as CYP2D6 Normal Metabolizer:

- The majority of experts agreed to downgrade CYP2D6*10 due to considerable reduction in activity. A CYP2D6*2x2/*10 genotype (AS 2.25), for example, would be categorized as a normal metabolizer with the assumption that *CYP2D6*10* function contributes very little to the overall function.

Other alleles that contain the *CYP2D6*10* function-defining SNP (100C>T; rs1065852)

Allele	Current CPIC function	AA change causing variants
*10	decreased	100C>T; 4180G>C
*49	decreased	100C>T; 1611T>A; 4180G>C
*54	decreased	100C>T; 2556C>T; 4180G>C
*65	decreased	100C>T; 2850C>T; 4180G>C
*72	decreased	100C>T; 3318G>A; 4180G>C
*37	uncertain	100C>T; 1943G>A; 4180G>C
*52	uncertain	100C>T; 3877G>A; 4180G>C
*64	uncertain	100C>T; 1023C>T; 4180G>C
*87	uncertain	14C>T; 100C>T; 4180G>C
*94	uncertain	100C>T; 3181A>G; 4180G>C
*95	uncertain	100C>T; 3334A>C; 4180G>C

Next steps

We are seeking feedback on the final CYP2D6 genotype to phenotype table. Please email any comments to contact@cpicpgx.org by September 20th. Please make sure to read the following carefully as it contains the rationale for each change made to the current system CPIC now uses.

Acknowledgements

- Andrea Gaedigk
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