Using the VerifyNow P2Y12 Assay to Optimize Dual Antiplatelet Therapy for Patients with Acute Stroke or Transient Ischemic Attack: Associations between P2Y12 Reaction Units and *CYP2C19* Polymorphisms

THE QUEEN'S MEDICAL CENTER

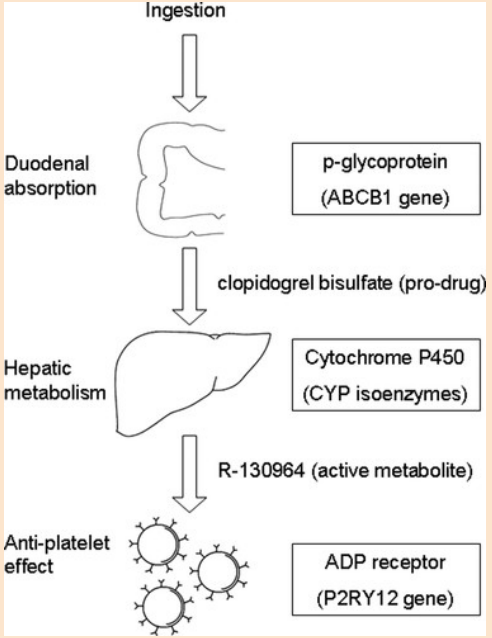
Jan Aurelio

Principal Investigator:
Dr. Stacy Brown, MD

Background

- Certain patients with acute ischemic stroke (IS) or transient ischemic attack (TIA) are treated with dual antiplatelet therapy (DAPT) to reduce risk for recurrent stroke
    - CHANCE and POINT trials

- DAPT in these trials consisted of clopidogrel and aspirin
    - Clopidogrel requires hepatic transformation to active metabolite via the *CYP2C19* enzyme



Anderson et al., 2010, *Stroke*

Background

- Polymorphisms exist for the *CYP2C19* gene
  - Some are loss-of-function (LoF), some are gain-of-function
  - LoF alleles are associated with lowered clopidogrel metabolism

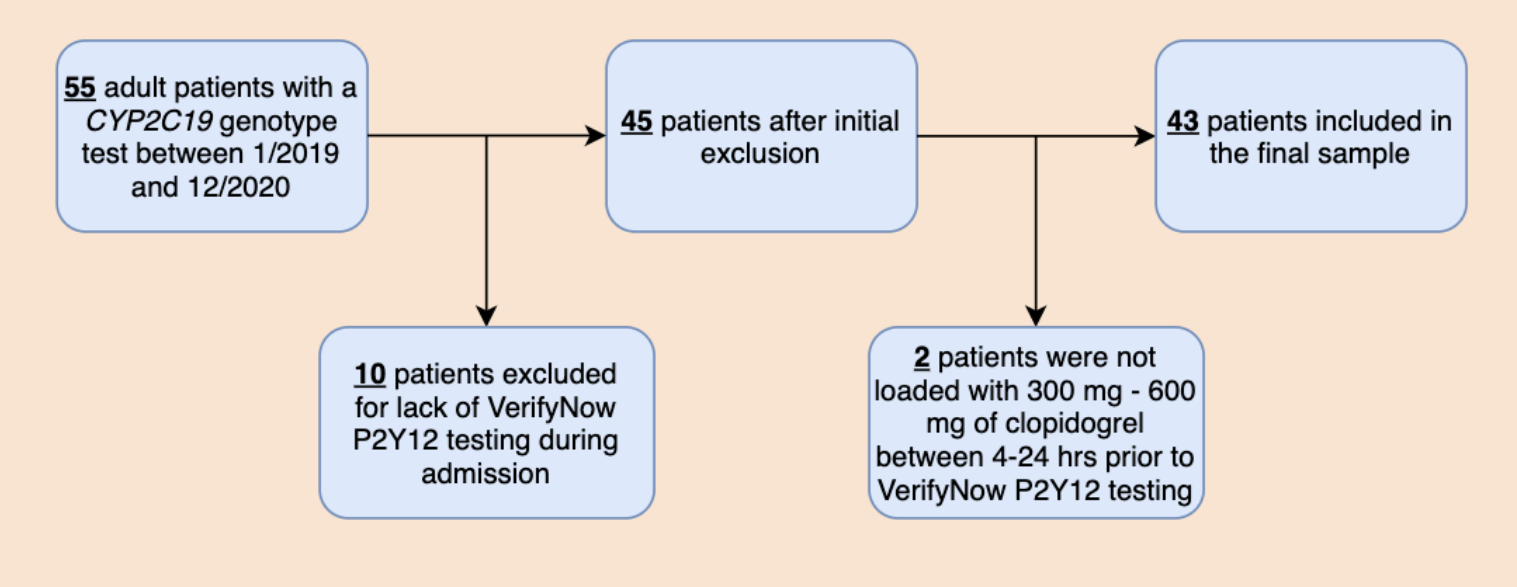
- VerifyNow P2Y12 assay measures platelet reactivity units (PRUs) in patients loaded with clopidogrel
  - High PRU (>180) suggest poor platelet inhibition from clopidogrel

Study Objectives

1) What are the sensitivity and specificity of a high post-load PRU for *CYP2C19* LoF genotype?

2) Are there comorbidities, concurrent medications, or racial/ethnic variables that may influence the association between high post-load PRU and *CYP2C19* LoF genotype?

Methods


55 adult patients with a CYP2C19 genotype test between 1/2019 and 12/2020
45 patients after initial exclusion
43 patients included in the final sample
10 patients excluded for lack of VerifyNow P2Y12 testing during admission
2 patients were not loaded with 300 mg - 600 mg of clopidogrel between 4-24 hrs prior to VerifyNow P2Y12 testing

Results

Table 1: Baseline Characteristics of Study Population, Stratified by Loss of Function Mutation in *CYP2C19*

	No LoF allele (n = 23)	At least one LoF allele (n = 32)	p-value
Age (mean [SD])	65.61 [11.42]	69.29 [11.14]	0.240
Male (%)	14 (60.9)	19 (61.3)	1.000
Race (%)			0.779
White	7 (30.4)	11 (35.5)	
Black	2 (8.7)	0 (0.0)	
Asian	10 (43.5)	14 (45.2)	
Native Hawaiian/ Pacific Islander	4 (17.4)	6 (19.4)	
P2Y12 Reaction Units (mean [SD])	139.45 [92.50]	193.83 [73.05]	**0.037**
Evidence of P2Y12 Inhibition (%)	14 (70.0)	8 (34.8)	**0.046**

Results

Table 2: Medical Characteristics of Study Population, Stratified by Platelet Receptor Blockade as Measured by P2Y12 Reaction Units

	No Platelet Receptor Blockade (n = 21)	Platelet Receptor Blockade (n = 22)	p-value
Age (mean [SD])	69.62 [9.51]	66.59 [12.05]	0.367
Male (%)	15 (71.4)	11 (50.0)	0.261
Diabetes Mellitus (%)			0.738
No	7 (30.4)	11 (35.5)	
Yes	2 (8.7)	0 (0.0)	
Pre-diabetes	10 (43.5)	14 (45.2)	
No data	4 (17.4)	6 (19.4)	
Methamphetamine Use (%)	0 (0.0)	2 (9.1)	0.490
No Cocaine Use (%)	21 (100.0)	22 (100.0)	N/A

Results

Table 2: Medical Characteristics of Study Population, Stratified by Platelet Receptor Blockade as Measured by P2Y12 Reaction Units (cont.)

	No Platelet Receptor Blockade (n = 21)	Platelet Receptor Blockade (n = 22)	p-value
Smoking Status (%)			0.332
Not a Smoker	9 (42.9)	14 (63.6)	
Current Smoker	3 (14.3)	3 (13.6)	
Former Smoker	9 (42.9)	5 (22.7)	
Excessive Alcohol Use (%)			0.135
No Excessive Use	20 (95.2)	19 (86.4)	
Binge Drinker	1 (4.8)	0 (0.0)	
Heavy Drinker	0 (0.0)	3 (13.6)	
Presence of LoF allele (%)	15 (71.4)	8 (36.4)	**0.046**

Results

Sensitivity and Specificity of VerifyNow P2Y12 Platelet Receptor Blockade (PRU < 180) for LoF Genotype

	Presence of LoF Allele	No LoF allele detected	Test Characteristic
No platelet inhibition (PRU > 180)	15	6	Sensitivity 65%
Platelet inhibition (PRU < 180)	8	14	Specificity 70%

Results

Sensitivity and Specificity of VerifyNow P2Y12 Platelet Receptor Blockade (PRU < 170) for LoF Genotype

	Presence of LoF Allele	No LoF allele detected	Test Characteristic
No platelet inhibition (PRU > 170)	17	6	Sensitivity 74%
Platelet inhibition (PRU < 170)	6	14	Specificity 70%

Results

Sensitivity and Specificity of VerifyNow P2Y12 Platelet Receptor Blockade (PRU < 150) for LoF Genotype

	Presence of LoF Allele	No LoF allele detected	Test Characteristic
No platelet inhibition (PRU > 150)	18	6	Sensitivity 78%
Platelet inhibition (PRU < 150)	5	14	Specificity 70%

Discussion

- In our study population, lack of platelet inhibition as defined by PRU > 180 has a sensitivity of 65% and specificity of 70% for *CYP2C19* LoF mutation

- Lowering the threshold for interpreting lack of platelet inhibition to PRU > 170 and 150 in our cohort increases the sensitivity to 74% and 78%, respectively, without affecting the specificity

- There was no significant difference in the race/ethnicity distributions between patients with and without LoF mutations
    - Previous studies have shown higher prevalence of LoF mutations in Asian populations, which may influence pre-test probability in clinical reasoning for some practices
    - In our study population, findings suggest that race/ethnicity are not reliable indicators of *CYP2C19* allele frequency

- Common medical comorbidities among patients undergoing testing do not associate with testing results

Study Strengths and Limitations

Strengths:

- Unique clinical protocol generated data to study test characteristics of the P2Y12 assay as a proxy for *CYP2C19* LoF genotype
- Patient population allowed inclusion of genetic information relevant to individuals of rarely studied ancestries

Limitations:

- Enriched sample precludes generalizability of findings
- Power limited by sample size

Conclusion

1) Among 43 patients who underwent *CYP2C19* genotyping at The Queen's Medical Center in 2019-2020, the VerifyNow P2Y12 assay was not a reliable proxy for *CYP2C19* loss of function mutation.

2) Larger studies are required to confirm test performance in a general patient population

3) Further studies will address the relationship between P2Y12 Reaction Units and clinical outcomes

Acknowledgements

Susan Asai

The Queen’s Stroke Team

Lori Tsue and the Queen’s Summer Research Internship

THE QUEEN'S MEDICAL CENTER

Citations

Anderson CD, Biffi A, Greenberg SM, Rosand J. Personalized Approaches to Clopidogrel Therapy: Are We There Yet? *Stroke*. 2010;41(12):2997-3002. doi:10.1161/STROKEAHA.110.594069