



**THE DISTRIBUTION OF ALTERED FORMS OF CYP2C19*2 ALLELE IN A
TRINIDADIAN POPULATION. IS CLOPIDOGREL THE RIGHT CHOICE?**

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ABSTRACT

Wyke M, Ramkissoon V, Griffith I, Ramnath M, Verma A, Carmichael C, Soo Ping Chow M, Mungrue K, O'Brien K and Mark A. University of the West Indies, Faculty of Medical Sciences, Department of Paraclinical Sciences Public Health & Primary Care Unit & Department of Preclinical Sciences, Biochemistry Unit Eric Williams Medical Sciences Complex, St Augustine Trinidad and Tobago. **Objective:** To determine the distribution of the CYP2C19*2 allele among Trinidadians. **Design and Method:** This is a cross sectional study among 100 patients attending primary health centers within the North Central Region of Trinidad. We used a stratified sampling technique in which there were three mutually exclusive subgroups South East Asians, Africans and mixed ethnicity. Subsequently systematic sampling was applied to each stratum to improve the representativeness of the sample. Hence the 100 subjects recruited for the study were 40 South East Asians, 40 Africans and 20 of mixed decent. Apart from baseline data which included age, gender and ethnicity DNA was assessed for the CYP2C19*2 allelic variant using a real time PCR method. **Results:** There was a high proportion (37%) of Allelic frequencies for CYP2C19*2 which was found to more common among South East Asian (47.5%, 95% CI 32.0-63.0) who also have high prevalence of CHD than Africans (45%, 95% CI 23.2-66.8) or people of mixed origin (95% CI 9.6-35.4). **Conclusion:** In conclusion we provide evidence that the prevalence of CYP2C19*2 mutations are high in our setting.

KEYWORDS: To determine the distribution of the CYP2C19*2 allele among Trinidadians.

INTRODUCTION

Coronary Heart Disease (CHD) defined in this study by the International classification of diseases ICD 9 codes 410–414 and ICD10 codes I20–I25 (ischaemic heart diseases), is the leading cause of death in the world and the leading cause of death in Trinidad.^[1,2] Coronary Artery Disease (CAD) and Acute Coronary Syndromes (ACS) greatly influence mortality rates in the Caribbean. The emergence of chronic non-communicable diseases particularly CHD was established since the mid-1900s continues to raise and challenge health services.^[3]

Clopidogrel a thienopyridine, P2Y₁₂ receptor inhibitor approved by the FDA in 1997^[4], is used as antiplatelet therapy to support primary percutaneous coronary intervention (PCI), which is currently the standard care for ST elevation MI (STEMI). A loading dose of a P2Y₁₂ receptor inhibitor should be given as early as possible or at the time of primary PCI to patients with STEMI and should also be given for 1 year to patients with STEMI

who receive a stent (bare-metal or drug eluting).^[5-10] However, the most recently published guideline for the management of patients with non-ST-elevation acute coronary syndromes (NSTEMI-ACS) by the American Heart Association and the American College of Cardiology^[11], specifies that it is reasonable to use the P2Y₁₂ inhibitor ticagrelor (Brilinta, Astra Zeneca) rather than clopidogrel in patients with NSTEMI ACS who undergo an early invasive or ischemia-guided strategy or who receive a coronary stent.

Clopidogrel is a biologically inactive pro-drug and is metabolically activated by the polymorphic enzyme 4'-hydroxylates S-mephenytoin known as CYP2C19. CYP2C19 is encoded by the CYP2C19 gene and its defective alleles account for poor metabolizers (PMs). PMs have been found to possess a mutated variant that can range from CYP2C19*2 to CYP2C19*8 out of a total of 21 variant alleles that exist for the CYP2C19 gene.^[12,13] The PM phenotype, is inherited as an

autosomal recessive trait. The main defective allele, CYP2C19*2, accounts for; 75–85% of alleles responsible for the PM phenotype in both Orientals and Caucasians.^[14]

Clopidogrel's efficacy is determined by functional variants of genes that code for these candidate cytochrome P450 (CYP) isoenzymes. Therefore, due to genetic variation and the existence of mutated CYP isoenzymes, clopidogrel's capacity for effectiveness shows wide interpatient variability.^[15] Data regarding in vitro metabolism and clinical outcomes suggest that the reduced-function CYP polymorphisms have a negative effect on enzymatic function and, therefore, negatively affect the conversion of clopidogrel to its active metabolite.^[16] The CYP2C19*1 allele is the normal/wild-type allele and exhibits full enzymatic activity. The CYP2C19*2 allele results in a total loss of enzymatic activity.

Recent studies have shown that, compared to non-carriers, carriers of a 'reduced-function' form of the CYP2C19 allele (PMs) experience significantly lower levels of the active metabolite of clopidogrel and reduced platelet inhibition and are at higher risk of experiencing major cardiovascular events.^[16] Those with a *2/*2 homozygous diplotype present as PMs in which there is low to absent enzyme activity whereas those who present with a *1/*2 heterozygous diplotype present as intermediate metabolisers where enzyme activity is intermediary.^[17] In both of these cases the therapeutic recommendations for acute coronary syndromes/percutaneous coronary intervention is therapy alternative to clopidogrel.^[17]

Studies have also shown the prevalence of the *2 alleles vary by ethnicity.^[18] The presence of the CYP2C19*2 polymorphism apart from resulting in ineffective treatment also provides a financial burden for patients who have to purchase the drug in this setting.

Therefore the aim of this study is to determine the distribution (homozygous and heterozygous.) of the CYP2C19*2 allele among Trinidadians.

METHODS

We used a cross sectional study design. The study population consisted of patients attending primary health care centers within the North Central Region of Trinidad. We randomly selected 100 patients (constrained by cost) without CHD or currently receiving drug therapy using first a stratified sampling technique and subsequently systematic sampling to improve representativeness. We used the distribution of the various ethnic groups in the population to weight the

proportion of each stratum and subsequently a sampling fraction of 1/10 to recruit subjects. Following informed consent a 5ml blood sample was taken from the antecubital fossa and placed in an EDTA container. The sample was transferred to the laboratory in wet ice at 4°C and stored at this temperature until DNA extraction. The genomic DNA was extracted using a commercial extraction kit (DNeasy blood and tissue kit, Qiagen, USA), which was stored at -20°C until PCR analysis. PCR was performed on a 50µl reaction mixture containing PCR buffer with 1.5mM MgCl₂, 0.2µM dNTPs, 0.2-0.8 µM of the specific forward and reverse primer, 1.25 unit of Taq DNA Polymerase (HotstarTaq Master Mix Kit, Qiagen, USA). The forward primer utilized for the CYP2C19*2 allele was 5'-AATTACAACCAGAGCTTGGC-3'.

The reverse primer used was 5'-TATCACTTTCCATAAAAAGCAAG-3'. The amplification condition was as follows: Initial denaturation at 94°C for 15 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 53°C for 40 seconds, extension at 72°C for 30 seconds. Final extension at 72°C for 5 minutes was performed (Zand).^[19] The amplification procedure was carried out in an Eppendorf Mastercycler PCR system.

The PCR products of the CYP2C19*2 allelic variant were digested with the restriction endonuclease SmaI as described by De Morais.^[20] Digested PCR products were analyzed using 3% agarose gels stained with ethidium bromide. Data was entered into an excel spreadsheet and later exported to SPSS (IBM Software Package for Statistical Analysis) for further analysis. Analysis included the percentage of patients with normal or mutated forms of the CYP2C19*2 allele as well as those with homozygous and heterozygous inheritance, as well as a comparison of the distribution of the allele by gender and ethnicity.

RESULTS

Allele frequencies for all populations tested are summarized in table 1.

Exactly 100 subjects, 36 males and 64 females were entered into study because of available resources to test for CYP2C19*2 allele. There were 40 South East Asians (SEA), 40 Africans and 20 subjects of mixed ethnicity. Of the 100 subjects tested, the CYP2C19*2 allele was present in 37 subjects (37%, 95% CI 27.5-46.5), occurring more commonly in males (15/36, 41.7%, 95% CI 25.6-57.8) than females (22/64, 34.4%, CI 22.8-46.0). Similarly it was more common among SEA (19/40, 47.5%) than subjects of mixed ethnicity (9/20, 45%) or Africans (9/40, 22.5%).

Table 1: Allele frequencies for CYP2C19*2 observed in African, South East Asian and Mixed Ethnicity populations (% , 95% CI in parenthesis).

	Africans	South East Asians	Mixed Ethnicity
Total No. Genotyped	40	40	20
Major Allele Homozygous	31 (77.5, 64.6-90.4)	21 (52.5, 37.0-68.0)	11 (55, 33.2-76.8)
Heterozygous	4 (10, 0.7-19.3)	8 (20, 7.6-32.4)	6 (30, 9.9-50.0)
Minor Allele Homozygous	5 (12.5, 2.2-22.8)	11 (27.5, 13.7-41.3)	3 (15, -0.7-30.7)
Minor Allele Frequency	22.5 (9.6-35.4)	47.5 (32.0-63.0)	45 (23.2 to 66.8)

CI, Confidence Interval.

Of the 9 Africans with the CYP2C19*2 mutation, 6 were male and 3 were female. Of the 6 males, 3 were heterozygous and 3 were homozygous for the CYP2C19*2 allele. Of the 3 females, 1 was heterozygous and 2 were homozygous for the CYP2C19*2 allele.

Hence there were 4 heterozygous (4/40, 10%) and 5 homozygous (5/40, 12.5%) positive Africans. The gender distribution of Africans with the wild type allele (31) was 7 male to 24 female.

Of the 19 SEA with the CYP2C19*2 mutation, 7 were male and 12 were female. Of the 7 males, 3 were heterozygous and 4 were homozygous for the CYP2C19*2 allele. Of the 12 female, 5 were heterozygous and 7 were homozygous for the CYP2C19*2 allele. The aggregate was 8 heterozygous (20% of the 40 tested) and 11 homozygous (27.5% of the 40 tested). The gender distribution of the SEA with the wild type allele (21) was 9 male to 12 female.

Of the 9 Trinidad of mixed ethnicity CYP2C19*2 mutation, occurred in 2 were males and 7 females. Of the 2 males, 1 was heterozygous and 1 was homozygous for the CYP2C19*2 allele. Of the 7 females, 5 were heterozygous and 2 were homozygous for the CYP2C19*2 allele.

This aggregated to 6 heterozygous (30% of the 20 tested) and 3 homozygous (15% of the 20 tested).

DISCUSSION

This is the first study of its kind on the prevalence of CYP2C19*2 mutations related to the genetic polymorphism of CYP2C19 in a Trinidadian population, a high prevalence setting for CHD. Thirty seven subjects (37%) were genotypically identified as PMs, with SEA having the highest percentage (47.5%). The risk of cardiovascular events is increased in patients who are PMs (carrying at least one CYP2C19*2 allele) despite receiving adequate doses of clopidogrel.^[16,21-23] This finding is consistent with similar studies reported elsewhere. In studies reported from Tamil Nadu, North India, Andhra Pradesh, Karnataka and Kerala which showed CYP2C19*2 frequencies of 38%, 30%, 33%, 39% and 31% respectively.^[24-26] Furthermore, the homozygous frequency was higher (27.5%) than those reported in India (7.4% - 17%).^[25-26] Heterozygous

frequency for Indo-Trinidadians (20%) was almost 20% lower than the lowest heterozygous frequency found in the Indian studies (42% - Andhra Pradesh).^[26]

Heterogeneity and homogeneity were observed to be at higher rates in Indo-Trinidadians (20%, 27.5%) than in the Afro-Trinidadian population (10%, 12.5%) while in the mixed population, heterogeneity (30%) was 10% higher than that of Indo-Trinidadians (20%) and homogeneity (15%) was more than 10% lower than that of Indo-Trinidadians (27.5%) but still slightly higher than that of Afro-Trinidadians (12.5%). (Figure 6).

In Indo-Trinidadians homozygous frequency showed a higher value (27.5%) to those found in the stated Indian studies which ranged from 7.4% - 17%.^[24-26] Heterozygous frequency for Indo-Trinidadians (20%) was almost 20% lower than the lowest heterozygous frequency found in the Indian studies (42% - Andhra Pradesh).^[26] The cause of these disparities in frequency may be the fact that this study's sample size (40) was significantly less than the sample populations used in the literature mentioned: 112 in Tamil Nadu, 121 in North Indian, 115 in Andhra Pradesh, 198 in Karnataka and 118 in Kerala. Therefore, because of the smaller sample size, the precision of the study may have been affected.

Compared to the studies of African Americans, Ethiopia, Tanzania, Zimbabwe, South Africa and Ghana, Afro-Trinidadians showed the highest frequency (22.5%) of CYP2C19*2 allele; The frequency found was very similar to that found in the Tanzanian studies of Herlin *et al.* (17.9%) and Dandara *et al.* (17.7%) and showed the highest correlation with the population study of Venda - 21.7%.^[33,37] Conversely, the CYP2C19*2 frequency found for Afro-Trinidadians was markedly higher than those found in the other studies of those of African continent where frequency ranged from 6% to 13.6%.^[18,27-31,33-36] Afro-Trinidadian heterozygosity for CYP2C19*2 was found to be low at 10% which showed similarity to the Ghanaian study by Kudzi *et al.* where 8% were heterozygous and the Tanzanian study of Bathum *et al.* where 16.4% were heterozygous.^[34,35] The studies Venda, Zimbabwe and Tanzania by Dandara *et al.* all showed significantly higher frequencies of heterozygosity at 32.9%, 19.1% and 29.9% respectively. The frequency of Afro-Trinidadian homozygosity (12.5%) was found to be significantly higher than the heterozygosity reported within the studies of the African

continent which had frequencies ranging from 1.5% to 5.3%. As stated before, these disparities exhibited can be attributed to the small population (40) that was studied for this research project.

In contrast to the literature reported, Afro-Trinidadian and Indo-Trinidadian homozygous frequency were found to be higher than their heterozygous frequency (Figure 6). In all studies observed, heterozygous frequency was found to be significantly higher than homozygous frequency.

It should be noted that on comparing the CYP2C19*2 frequency between those of African descent and those of Indian descent, Indo-Trinidadians showed a distinctly higher prevalence of the allele which correlated with the studied literature.

Due to the high frequency of CYP2C19*2 revealed among Afro-Trinidadians, Indo-Trinidadians and those of admixture, it can be said that treatment clopidogrel is not the most effective option for Trinidad.

A major limitation was the classification of ethnic groups into SEA and Africans as it can sometimes be difficult to ascertain. In addition the study had to be restricted to a 100 subjects due to cost. Notwithstanding, the effect size was large thus providing evidence for the first time that the prevalence of CYP2C19*2 mutations are similar to studies done with larger samples. The implication of our findings for clinical practice includes genotyping prior to clopidogrel therapy and the use of Ticagrelor was an alternative.

In conclusion we provide evidence that the prevalence of CYP2C19*2 mutations are high in our setting. The cytochrome p450 enzyme (CYP2C19) is responsible for metabolizing a large number of commonly prescribed medications such as omeprazole, clopidogrel, phenytoin, imipramine, indomethacin, and warfarin. Several articles have reported an increase in adverse outcomes in both poor and intermediary metabolizers treated with standard doses of clopidogrel.^[16,21-23,37-48] Two large meta-analyses have confirmed these findings.^[49,50] There was sufficient accumulated evidence regarding the correlation of CYP2C19 poor and intermediate metabolizer phenotypes with adverse outcomes for the FDA to place a “box warning” in the package insert informing clinicians of the availability of genetic testing.

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