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Clinical Implications of Genetic Admixture in Hispanic Puerto Ricans: Impact on the Pharmacogenetics of *CYP2C19* and *PON1*

Jorge Duconge[1], Odalys Escalera[1], Mohan Korchela[2] and Gualberto Ruaño[2]
[1]University of Puerto Rico School of Pharmacy, Medical Sciences Campus,
Pharmaceutical Sciences Department, San Juan,
[2]Genetic Research Center, Hartford Hospital, Hartford,
[1]Puerto Rico
[2]USA

1. Introduction

Antiplatelet therapy with clopidogrel (Plavix®) is now considered a cornerstone of cardiovascular medicine. Clopidogrel resistance is an emerging clinical entity with potentially severe consequences such as recurrent myocardial infarction (MI), stroke, or death. Since its initial description, multiple investigators have confirmed the phenomenon of clopidogrel resistance (Dziewierz et al., 2005; Gurbel & Tantry, 2007; Mega et al., 2009; Mobley et al., 2004; Müller et al., 2003). The prevalence of clopidogrel non-responsiveness has been reported at 5–44% in other populations (Gurbel & Tantry, 2007). Although the occurrence of clopidogrel resistance is multi-factorial, it has been associated with a *CYP2C19*- and *PON1*-mediated[1] patient's metabolic inability to generate sufficient active metabolite to arrest platelet reactivity. Rapid and accurate detection of clopidogrel resistance in Hispanic Puerto Rican patients remain unsolved as new bedside genetic tests are developed.

In a recent GWAS analysis, it was estimated that up to 83% of individual variance in response to clopidogrel might be attributable to genetic effects (Shuldiner et al., 2009), but the gene variants investigated thus far explain only a minor proportion of such response variability (Holmes et al., 2010; Hulot et al., 2010). The high heritability of clopidogrel response but relatively weak prediction by existing proposed genetic markers argues for the involvement of some as yet undiscovered genetic factors. The *CYP2C19*2* allele was reported to account for only 12% of the variability in ADP-stimulated platelet response to clopidogrel (Shuldiner et al., 2009). The *PON1* Q192R polymorphism explained 72.5% of clopidogrel response variability in individuals of European ancestry (Bouman et al., 2011). However, a previous GWAS in a cohort of related healthy subjects of Amish descent provided no evidence for an association of the *PON1* gene region with the platelet response to clopidogrel (Shuldiner et al., 2009).

Due to its remarkable heterogeneity and trichotomous ancestral genetic admixture, the Puerto Rican population may significantly differ from other earlier pharmacogenetically

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[1] *CYP2C19* stands for Cytochrome P450 isoform 2C19 (family 2, subfamily C, polypeptide 19) gene; *PON1* stands for Paraoxonase 1 gene.

characterized populations with respect to the frequency, distribution and combination of allelic variants in genes associated with drug response and diseases (González-Burchard et al., 2005; Suarez-Kurtz et al., 2006). We have published a physiogenomic analysis to infer structure and ancestry in the Puerto Rican population. The Puerto Rican sample was found to be broadly heterogeneous, with three main clusters reflecting the historical admixture from Taino Amerindians, West-Africans, and Iberian European ancestors (Ruaño et al., 2009). Our results matched previously published estimations of Puerto Rican admixture that were ascertained using more traditional ancestral genetic markers (Bertoni et al., 2003; Bonilla et al., 2004a, 2004b; Choudhry et al., 2006; Hammer et al., 2006; Hanis et al., 1991; Martinez-Cruzado et al., 2001, 2005). The study provided a set of 384 physiologically informative SNPs from 222 cardio-metabolic and neuro-endocrine genes that can be used to facilitate the translation of genome diversity into personalized medicine and control for admixture in Puerto Ricans. The observed large variance in admixture proportions suggested that this population is ideal for admixture matching studies.

Admixture is of great relevance to the clinical application of pharmacogenetics and personalized medicine, but unfortunately these studies have been scarce. As physiogenomic-guided multi-gene models are developed to predict drug response, the range of possible allelic combinations in the Puerto Rican population is certain to exceed that in populations without admixture. In addition, the allele frequencies for both the *CYP2C19* and *PON1* candidate genes in this population have not been fully characterized. Accordingly, we investigated whether a correlation between overall genetic similarity and *CYP2C19* and *PON1* genotypes could be established in the study population. This chapter also provides valuable evidence on the importance of controlling for admixture in pharmacogenetic studies of admixed populations like Puerto Ricans. Indeed, we will discuss how known or cryptic population stratification can have a strong confounding effect on further clinical association analysis. Finally, the necessity to utilize results of our admixture analysis to parameterize population structure appropriately and account for it as a covariate in the corresponding association studies will also be considered.

2. Methods

2.1 Specimens

100 human genomic DNA samples (40-60 ng/μL) were extracted and purified from existing dried blood spots on Guthrie cards supplied by the Puerto Rico Newborn Screening Program (PRNSP), where >95% of Puerto Rican newborns are screened for common hereditary diseases. Accordingly, sample analysis in this survey (*protocol #A4070107*) was exempt from IRB review under FDA and OHRP guidelines based on category 4, 45CFR46.118. A controlled stratified-by-region random sampling protocol was followed, taking into consideration the percentage of birth at each region across the Island of Puerto Rican based on the 2004 national register of total births. Two samples were discarded from final analysis due to poor quality.

2.2 Array

The extracted genomic DNA samples were genotyped on a physiogenomic (PG) array detecting 384 SNPs from 222 cardio-metabolic and neuro-endocrine genes spanning their entire genome (Ruaño et al., 2005a; Ruaño & Windemuth, 2006). Since the PG-array was constructed such that it covers all common variations in the general population for

pharmacologically relevant cardio-metabolic pathways, virtually any set of candidate genes within these pathways can be tested from the resulting dataset without any further assay. Consequently, genotypes associated with the clinically relevant *CYP2C19**2 (*rs*4244285; splicing defect G681A SNP) and nonsynonymous *PON1* (*rs*662; p.Q192R) polymorphisms were loaded from the derived database. Instead, array translation was used to impute genotyping related to *CYP2C19**3 (*rs*4986893; stop codon G636A SNP) with high accuracy, given its strong linkage disequilibrium with SNP *rs*3758581. Wild-types were assigned as a result of the absence of such SNPs. The PG array has been tested on nearly 5,000 patients and has been successfully applied in cardiovascular and neuropsychiatric pharmacogenetic research, resulting in ten publications (de Leon et al., 2008; Liu et al., 2009; Ruaño et al., 2005b, 2007a, 2007b, 2008, 2009, 2010; Seip et al., 2008; Windemuth et al., 2008). Careful manual analysis was performed on the alignments underlying the genotype calls using GenCall 6.1.3.24 and 50 SNPs with even a slight degree of uncertainty about calling accuracy were not included in the analysis, leaving 332 SNPs from 196 genes. Genotyping was accomplished using Illumina® BeadArray™ technology (Oliphant et al., 2002).

2.3 Clustering and statistical analysis

Analysis of the results using the STRUCTURE v2.2 software package was used to cluster those subjects with similar genetic profiles (Falush et al., 2007; Pritchard et al., 2000). Hierarchical clustering algorithm was blind as to ethno-geographic ancestry. A detailed explanation of the analytics underlying the clustering of the samples and the hierarchical stratification by allelic dissimilarities to infer population structure, as well as a full list of the genes and SNPs in the PG array, is provided elsewhere in our physiogenomic analysis study (Ruaño et al., 2009). Allele frequencies, f, linkage disequilibrium (LD), and haplotype structure were individually determined for all loci. Wright's F-statistic was calculated for each locus from the observed total heterozygosity $H_T=N_{hz}/N$ and the subpopulation heterozygosity $H_S=2f\cdot(1-f)$, assumed under Hardy-Weinberg equilibrium (HWE), as $F_{ST}=(H_T-H_S)/H_T$. A t-test was performed to see whether the average Fst across all loci is different from zero. Departure from HWE were estimated under the null hypothesis of the predictable segregation ratio of specific matching genotypes (p>0.05) by use of χ^2 goodness-of-fit test with one degree of freedom. In addition, χ^2 test was also used to compare observed allele frequencies within each sector in the corresponding dendrogram with expected frequencies given the overall allelic ratios, at 5% of significance.

3. Results

Results are presented in **Table 1**, and graphically in **Figure 1** by hierarchical dendrograms to illustrate the population structure as represented by allelic dissimilarity (i.e., genetic distance). There appear to be three main sectors, from left to right: PR126–PR341; PR321–PR69 and PR97–PR175. There are also two smaller sectors between the three main sectors. One pair of samples (PR26 and PR92) shows an unusually low allelic dissimilarity, suggesting relatedness. However, due to anonymousness of collected samples, we were unable to further investigate this issue. Those individuals who shared many polymorphisms were grouped together in one "sector" while those with whom they had much more genetic dissimilarities lie on the other side of the "phylogenetic tree' map; thus greater distances denoting lesser degrees of common ancestry.

Gene	SNP	Ch	Location	N^a	$Alleles^b$	MAF (%)	Genotypes			Fst
							WT	*HET*	*MUT*	
CYP2C19	*rs*4244285 (*CYP2C19*2*)	10	96541616	98	**G**A	14.8	52	17	2	0.042
CYP2C19	*rs*3758581 (*CYP2C19*3*)	10	96602623	95	**G**A	3.5	66	5	0	0.017
PON1	*rs*662	7	94937446	96	A**G** (T/C)	45.1	22	34	15	0.174

Table 1. Genes, allele and genotype frequency distributions of clopidogrel-associated polymorphisms in the representative sample of Puerto Ricans analyzed by PG-array. Ch column display the chromosome on which the SNP can be found. Location column contains the chromosome location for each SNP given by the NCBI Entrez SNP database, build 36.3 reference assembly. MAF stands for minor allele frequency. [a]Total number of samples analyzed by PG-array ranged between 95 to 98. However, only 71 samples were used for STRUCTURE clustering analysis due to incomplete combinatorial genotyping data. Only those samples having a strong association with one of the three clusters were finally included in the superimposition analysis. [b]Ancestral allele in bold. First allele is the reference one.

To determine if there are relationships between genetic clusters defined by STRUCTURE analysis and clopidogrel-related genotypes, we superimposed the individual genotypes for *CYP2C19* and *PON1* on the dendrogram. **Figures 1**, panel A and B, show less than 98 cases (i.e., some lanes in the genetic distance map do not have identified ancestry) due to incomplete combinatorial genotyping data during the initial PG array analysis or because the individual residing in the site did not meet a categorical criterion of proximity (> 0.20), which precluded definitive assignment to any of the three STRUCTURE clusters (ancestry). Furthermore, only those samples having a strong association with one of the three clusters were included in the superimposition analysis, yielding 71 total subjects. Notice that only 52 out of these 71 analyzed cases were actually located within any of the three identified sectors in the genetic distance dendrograms seen in **Figures 1**. The other 19 cases, although located outside the sectors, were also included in the analysis because they all had a significant contribution from one of three ancestries (i.e., Taino Amerindians, Iberian Caucasians or West-Africans), as denoted by their ancestral coefficients. To this purpose, each of these 19 individual was allocated to the sector that best matched with his/her greater ancestral coefficient. The distribution of samples by sectors in the corresponding genetic distance dendrograms was then as follows: 20 samples in sector 1; 27 in sector 2 and 24 in sector 3.
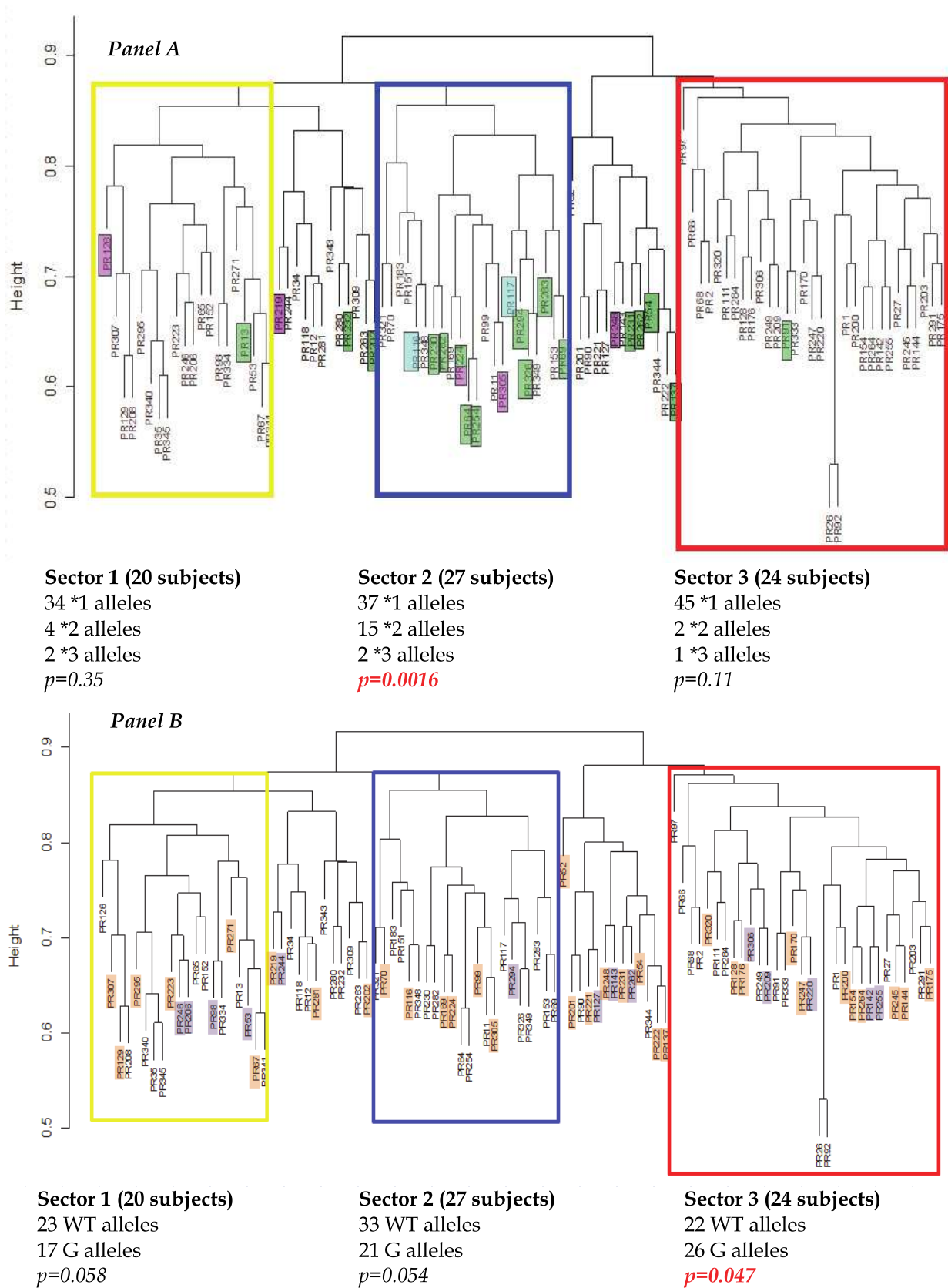
Sector 1 (20 subjects)
34 *1 alleles
4 *2 alleles
2 *3 alleles
p=0.35

Sector 2 (27 subjects)
37 *1 alleles
15 *2 alleles
2 *3 alleles
p=0.0016

Sector 3 (24 subjects)
45 *1 alleles
2 *2 alleles
1 *3 alleles
p=0.11

Sector 1 (20 subjects)
23 WT alleles
17 G alleles
p=0.058

Sector 2 (27 subjects)
33 WT alleles
21 G alleles
p=0.054

Sector 3 (24 subjects)
22 WT alleles
26 G alleles
p=0.047

Fig. 1. **Panel A**. Individual *CYP2C19**2 and *3 genotypes overlaid on the genetic distance dendrogram for the samples from the Puerto Rican population (dendrogram taken from

previously published physiogenomic population analysis [21]). Green boxes are depicting those individuals having a single *CYP2C19**2 polymorphism (G/A); Blue-colored boxes represent double carriers for *CYP2C19**2 (A/A); whereas, purple-colored rectangles indicate single carriers of *CYP2C19**3 (A/G). Double-colored rectangle (subject PR224) highlights the one having the *2/*3 genotype. The *CYP2C19**3 G→A polymorphism is in high linkage disequilibrium with *rs*3758581, which has been associated with a significant decrease in clopidogrel activation per allele and, therefore, resistance phenotype (non-responder) to standard dose. **Panel B**: Individual *PON1* Q192R genotypes overlaid on the genetic distance dendrogram for the samples from the Puerto Rican population. Yellow color represents single carriers; purple color denotes double carriers of this polymorphism (G allele). *p*-values were calculated by a χ2 test comparing observed allele frequencies with expected frequencies given the overall allelic ratios.

By previous STRUCTURE clustering analyses, the dendrogram sectors 1, 2 and 3 in **Figure 1** correspond to Taino Amerindian, Iberian Caucasian, and West-African heritage, respectively (Ruaño et al., 2009). Indeed, sector 1 bears a concentration of samples assigned to cluster 2 (Amerindians) in the STRUCTURE plot. Sector 2, in the middle, is disproportionately rich in samples corresponding to cluster 3 (Europeans). Sector 3, to the right, is clearly enriched with cluster 1 samples (Africans). For the 71 cases with complete genotypes, the *CYP2C19**2 allele frequency was 14.8%, whereas the *3 allele frequency was 3.5%. Although slightly higher, these findings are consistent with early reports in 22 Latin Americans (Mexicans and Puerto Ricans) from the 1,000 Genomes Phase I selection, where the *CYP2C19**2 and *3 minor allele frequencies were 13.6% and 1.7%, respectively. Regarding to *CYP2C19**2 polymorphism, we identified 52 homozygous for the wild-type allele G, 17 heterozygous and 2 double carriers of the variant allele A. However, the analysis for *CYP2C19**3 revealed 66 wild-types, 5 single carriers of the variant allele, but none double carrier of this polymorphism in the study population. Sectors 1 and 3 in Figure 1, panel A, show *CYP2C19**2 allele frequencies of 10% (4 out of 40) and 4.16% (2 out of 48), respectively, as compared to 27.8% (15 out of 54) in sector 2. Statistical analyses to compare each sector to the overall allelic ratios, revealed that sector 2 (in the middle of the genetic distance dendrogram, **Figure 1**-panel A), showed a frequency of *CYP2C19**2 that was 4-fold higher than the rest of the population (*p*=0.0016). Likewise, carrier prevalence in sector 2 (48.15%) was significantly greater than that observed in the other two sectors (i.e., 20 and 8.3%, respectively).

Sector 2 is associated with Iberian European heritage (Ruaño et al., 2009). According to the HapMap project dataset (International HapMap Consortium, 2005), the minor allele frequency for *CYP2C19**2 is 14.3% in 223 Caucasians who are Utah residents with Northern and Western European ancestry from the CEPH collection (HapMap-CEU; data release#28, Phase II + III). Similarly, the *CYP2C19**2 allele frequency was 13% for 261 Europeans in the 1,000 Genomes Phase I selection (Amigo et al., 2008). The present study reports a relatively inflated minor allele frequency of 27.8% for the *CYP2C19**2 variant in sector 2 (primarily Caucasian), consistent with the possible interpretation that Puerto Ricans in this sector reflect an increased admixture with Asians (i.e., Taino Amerindians). The link between Amerindians and Asians originates with the "Bering Strait" theory (Jennings, 1979). According to previous reports, there seems to be a very high frequency for the *CYP2C19**2 variant in East Asian populations, ranging from 27-32.5% (Amigo et al., 2008).

Likewise, the observed minor allele frequency of 4.16% in sector 3 is lower than expected for a population of purely African ethno-geographic origin, no surprise given the heterogeneity

of the Puerto Rican population. The reported *CYP2C19**3 allele frequencies of 5% for sector 1, 3.7% for sector 2 and 2.1% for sector 3 compare to the expected HapMap values of 5.6%, 1.7% and 0.3%, respectively. The higher than expected frequencies observed in sector 2 and 3 seem to be a direct consequence of significant admixture with Asian ancestry in individuals assigned to these clusters. Interestingly, the NCBI webpage reports a *CYP2C19**3 minor allele frequency of 2.1% in 24 individuals of self-described African-American heritage (AFR1, SNP500Cancer project, this database is an integral component of the NCI Cancer Genome Anatomy Project, available at http://cgap.nci.nih.gov), which is a population with certain genetic admixture (Packer et al., 2006). This value matched the observed allele frequency in sector 3, a cluster of majorly African ancestry.

With respect to the *PON1* gene (**Table 1**), the overall allele frequency was 45.1% (34 heterozygous and 15 homozygous for the variant allele), which is stratified by clusters as 42.5%, 38.9% and 54.2% in sectors 1, 2 and 3, respectively (**Figure 1**-Panel B). The observed prevalence for this variant is consistent with early reports in both 22 Latin America inhabitants (Mexicans and Puerto Ricans; 1,000 Genomes Phase I selection) and 58 Mexican descendants who reside in Los Angeles, California (HapMap-MEX; HapMap project dataset, release#28, Phase II + III), where the *PON1* minor allele frequencies were estimated to be 47.7% and 50%, respectively. The minor allele frequency in the general population ranges from 42.2 to 47.9%. However, when comparing observed allele frequencies in each of the three sectors (i.e., 1=Amerindian; 2=Caucasian; 3=West African) with the HapMap and 1,000 Genomes Phase I project values, the results are showing a significantly higher than expected prevalence of this *PON1* polymorphism. In sector 1, there was a minor allele frequency of 42.5% as compared with the HapMap report of about 36% for the Han Chinese of Beijing (International HapMap Consortium, 2005) and the 36.2% in East Asians from 1,000 Genomes Phase I selection (Amigo et al., 2008).

Furthermore, the allele frequency in sector 2 was 38.9% (13 heterozygous and 4 double carriers), considerably higher than the reported 29% for Caucasian residents of Utah, documented in the HapMap database (International HapMap Consortium, 2005), and the 28.4% presented by the 1,000 Genomes Phase I selection for Europeans (Amigo et al., 2008). Additionally, in sector 3 the allele frequency was 54.2% (12 heterozygous and 7 double carriers), which is by far a higher prevalence versus the HapMap allele frequency of 27.9% for the Nigerian YRI population or the 23.4% in Africans of the 1,000 Genomes Phase I selection (Amigo et al., 2008; International HapMap Consortium, 2005). Statistical analyses to compare each sector to the overall allelic ratios, revealed that sector 3 (right-most portion of the genetic distance dendrogram, **Figure 1**-panel B), showed a frequency of *PON1* Q192R that was only marginally different than the rest of the population (p=0.047).

Greater admixture and heterogeneity in the Puerto Rican population as compared to the African-American population or certainly the Yoruba population allow for the presence of the twenty-six *PON1* variant allele observed in the twenty-four individuals in sector 3 (**Figure 1**-Panel B) to have come from partial Amerindian ancestry, as no individual in that sector was of purely West-African descent (Ruaño et al., 2009). The observed trends may also be an artifact of chance given the relatively small sample size. No statistically significant deviations from HWE were found with respect to the distribution frequencies of either *PON1* or *CYP2C19* polymorphisms. Since no departures from HWE were observed and considering that our study cohort is island-wide, chosen by a controlled, stratified-by-

region, representative sampling from the Puerto Rican population, we can expect the observed frequencies of the *PON1* and *CYP2C19* polymorphisms to be representative for the rest of this population.

4. Discussion

In this work, we examined two clinically relevant *CYP2C19* gene polymorphisms (i.e., *CYP2C19**2 and *CYP2C19**3) and the emerging *PON1* variant in a representative sample of the Puerto Rican population. Our findings suggest a significant burden of loss-of-function *CYP2C19* and *PON1* carriers within the Puerto Rican population. Based on observed prevalence of functional *CYP2C19* and *PON1* polymorphisms in Puerto Ricans, and the postulated role of the enzymes encoded by these genes in the clopidogrel activation (Bouman et al., 2011; Mega et al., 2009; Shuldiner et al., 2009), we hypothesized that about 30-40% of Puerto Ricans may be non-responders to clopidogrel and such resistant patients may be at particular risk for short-term thrombo-ischemic complications including periprocedural infarction and early stent thrombosis.

Because thromboembolism is a major risk of cardiovascular disease, genotyping for clopidogrel's response-associated gene *CYP2C19*, and *PON1* to a lesser extent, have been advanced as desirable in Caucasians to improve patient's clinical outcomes (Bouman et al., 2011; Holmes et al., 2010; Hulot et al., 2010; Mega et al., 2009; Shuldiner et al., 2009). However, heterogeneity and admixture may preclude their full application in other populations such as Puerto Ricans. Accordingly, there is an urgent need for ascertaining admixture adjustments that may prove clinically useful in Puerto Ricans and other Hispanic groups. Indeed, the richer genetic variation in Puerto Ricans is likely to contribute substantially to a wider variation in response to clopidogrel treatment, a component that will be missed by traditional studies in homogeneous populations. This addressable oversight is of great concern, since it will tend to exacerbate the healthcare disparity already experienced by Hispanics in USA.

The population of the Americas carries a genomic legacy resulting from the continents' native inhabitants, European colonization and African slavery. In those parts of the Americas where admixture took hold, the populations manifest the combined anthropological heritage in their genomes, lifestyle, diet, and even socioeconomic status. Admixture is of great relevance to the clinical application of the pharmacogenetic-guided personalized medicine paradigm, but unfortunately these studies have been scarce in Puerto Ricans. We have performed pivotal physiogenomic studies on the Puerto Rican population by using an array of 384 SNPs in 222 cardio-metabolic and neuro-endocrine genes coding for relevant pharmaceutical targets. According to our findings, the Puerto Rican population represents different admixtures of 3 major ethno-geographic groups (i.e., Taino-Amerindians, Iberian-Europeans and West-Africans). Notably, each subject in the study population was a 'genetic mosaic', with contributions from each of these three clusters, but in widely different proportions.

Consequently, admixture in the Puerto Rican population exists in the form of a continuous gradient with varying levels of mixture that results in a rich repertoire of combinatorial genotypes for key pharmacological pathways such as the one associated with CYP2C9 and PON1 activity in humans. This phenomenon may explain discrepancies between observed

and expected genotype and allele frequencies across the three sectors in the genetic distance dendrograms (**Figure 1**-panel A and B), particularly with respect to other homogeneous (parental) populations previously characterized through the HapMap, SNP500Cancer and 1,000 Genomes projects. Moreover, the diversity observed in the genetic structure within African populations from varying regions of this continent (Tishkoff et al., 2009) may also contribute to explain the higher than expected prevalence of *PON1* variant allele at sector 3 (**Figure 1**-panel B) in our study population, which is an Afro-Caribbean population, as compared to continental Africans like Yoruba from Nigeria (HapMap-YRI). Overall, our findings further substantiate the argument for including admixture as a critical covariant in predicting clopidogrel response within heterogeneous populations, but also render the Puerto Rican population a good resource to develop DNA-guided systems for clinical management of thromboembolic disorders, an urgent medical need not only in Hispanics but also in the population at large.

Recently, we provided valuable evidence on the importance of controlling for admixture when conducting pharmacogenetic studies of warfarin (Coumadin®) in the Puerto Rican population and stressed the argument for incorporating admixture-matching in order to probe variations in warfarin response across different stratum within the population (Villagra et al., 2010). In this study we postulated that interindividual variations in ancestral contributions of Puerto Ricans may help explain the observed poor performance and low predictability of DNA-guided warfarin dosing algorithms derived in other populations. Admixture introduces distinct levels of population sub-structure or stratum, with marked variations in individual ancestry among the members of a particular population or ethnic group, depending on the dynamics of the process. Accordingly, any extrapolations of clinically relevant pharmacogenetic data from non-admixed to admixed groups will be plagued with uncertainty as exemplified by Suarez-Kurtz (Suarez-Kurtz, 2005). Consequently, the predictive power of previously published DNA-guided algorithms for admixed populations like Puerto Ricans is expected to be inaccurate, if not inadequate.

A previous study by Perini and co-workers (Perini et al., 2008) stated that self-reported race, using labels defined by skin color as a proxy for ethnicity, was not a reliable indicator of effective anticoagulation therapy in admixed Brazilians. What proved useful, instead, was a precise knowledge of individual ancestral proportions so as to place the patient on a continuum between "black" and "white". The utility of this model was verified later on a separate cohort in the same population (Vargens et al., 2008). To control for possible marginal effects of ancestry on drug response, investigators of the GALA and SAGE projects included genetic ancestry as an independent variable in the regression model used to test association between *IL6R* SNPs and bronchodilator effect. Interestingly, the mean bronchodilator response for the pharmacogenetic interaction increased with increasing amounts of Native American ancestry; whereas, the drug response among asthmatic patients for the same pharmacogenetic interaction decreased with increasing amounts of European ancestry (Corvol et al., 2009). Differences observed among Mexican, Puerto Ricans and African-Americans were thus explained by the different proportions of Native American and European ancestries in these three ethnically diverse populations. Recently, Bryc and co-workers found evidence of a significant sex bias in admixture proportions of Hispanics that is consistent with disproportionate contribution of European male and Amerindian or African female ancestry to present populations (Bryc et al., 2010). These

authors also suggested that future genome-wide association studies in Hispanics will require correction for local genomic ancestry at a sub-continental scale.

Population stratification by admixture is a well-known confounder in pharmacogenetic association studies of candidate gene to complex traits, including drug response (e.g., clopidogrel). In this context, it might be difficult to find a matching control for an individual with diverse ethnic origins; therefore, we will be forced to rely on multivariate adjustment models. That is, rather than allocate the subject to a single stratum in the analysis, we recommend to construct a covariate for each stratum, giving the corresponding ancestral proportion derived from our admixture-driven clustering analysis, and then include these covariates as adjustment factors in a multiple regression model for clopidogrel association studies in Puerto Ricans. In doing so, we will be able to parameterize admixture-derived population structure appropriately and account for it as a covariate in the corresponding association studies in order to minimize the effect of population stratification.

We call these covariates "admixture indexes", which we believe are indispensable to assure that pharmacogenetic research can be pursued in Hispanic populations. From a key methodological perspective, the wider genetic variation found in our population for these markers broaden the reach and enhance the statistical sensitivity of tests for the effects of that variation on clopidogrel response. At the same time, the admixture index could become indispensable for the globalization of patho- and pharmacogenetic research beyond the Americas, to Africa, Asia and Europe, the continents whose populations contributed to the admixture in the first place.

In the context of admixture matching, if a resistant allele is more common in one of the ancestral populations, then non-responders to clopidogrel will share a greater level of ancestry from that population around the locus as compared with responders. In future studies, we expect to generate a detailed admixture map in the Puerto Rican population at very high resolution using all 1.2 million SNPs from a total genome (TG) array. Admixture studies at this resolution afford delineation of candidate genes for pharmacogenetic traits related not only to clopidogrel, but also to other drugs commonly used to treat cardiovascular conditions of high prevalence in Hispanics.

A major advance in healthcare would be a transition from the current empirical approaches in drug therapy to a genetically predictive framework for determining the individual patient's response to medicines. Accordingly, an understanding of how human genetic diversity and admixture in Hispanics is structured is not only of anthropological importance, but also of medical relevance. In addition, it is important to recognize that some minority groups in the U.S. (e.g., Hispanics) might be underrepresented in typical clinical pharmacogenetic trials with respect to the real impact of these groups on the current U.S. population. Because of the heterogeneity and extensive admixture of the Puerto Rican population, extrapolation on a global scale of data derived from well-defined ethnic groups (i.e., Caucasians) is clearly not applicable to the majority of Puerto Ricans.

5. Conclusion

In conclusion, we have established clinical correlations between overall genetic similarities and both *CYP2C19* and *PON1* genotypes in a representative sample of the Puerto Rican population. To this purpose, the major *CYP2C19* and *PON1* polymorphisms were

interrogated in 98 genomic DNA specimens using the physiogenomic (PG)-array that also inferred population structure and admixture pattern. Individual *CYP2C19* and *PON1* genotypes were visually overlaid atop three major sectors of a genetic distance dendrogram that was constructed by clustering subjects with similar genetic profiles. Results suggest that the observed inter-individual variations in ancestral contributions will have significant implications for the way each Puerto Rican responds to antiplatelet therapy with clopidogrel. Our findings also provided valuable evidence on the importance of parameterizes the population structure in order to account for admixture as a covariate in pharmacogenetic association studies for clopidogrel in Hispanic Puerto Ricans.

Rather than ignoring admixture, pharmacogeneticists should consider it as starting point for better understanding the underlying basis of the observed wider variability in drug responses among patients of mixed populations. Such understanding provides the opportunity to develop strategies for leapfrogging the healthcare standards in these populations.

6. Acknowledgement

7. Statement

A glossary of genetic terminology is maintained by the National Human Genome Research Institute at www.genome.gov/glossary.cfm.

8. Keywords

Admixture; Puerto Ricans; Pharmacogenetics; Clopidogrel; *CYP2C19*; *PON1*

9. Disclosure

Dr Ruaño is founder and President of Genomas, Inc. Mr. Kocherla is full-time employee of Genomas, Inc. The rest of the authors have no potential conflicts of interest to disclose.

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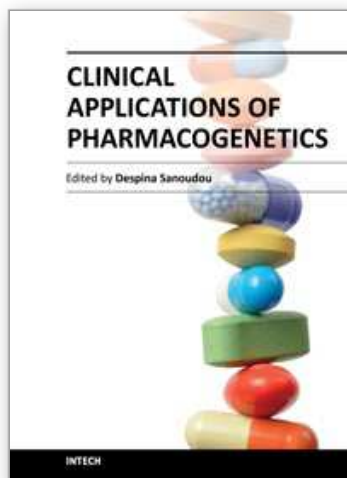
Clinical Applications of Pharmacogenetics

Edited by Dr. Despina Sanoudou

The rapidly evolving field of Pharmacogenetics aims at identifying the genetic factors implicated in the inter-individual variation of drug response. These factors could enable patient sub-classification based on their treatment needs thus expediting drug development and promoting personalized, safer and more effective treatments. This book presents Pharmacogenetic examples from a broad spectrum of different drugs, for different diseases, which are representative of different stages of evaluation or application. It has been designed so as to serve both the unfamiliar reader through explanations of basic Pharmacogenetic concepts, the clinician with presentation of the latest developments and international guidelines, and the research scientist with examples of Pharmacogenetic applications, discussions on the limitations and an outlook on the new scientific trends in this field.

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