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Genome-wide and candidate gene approaches of clopidogrel efficacy using pharmacodynamic and clinical endpoints - Rationale and design of the International Clopidogrel Pharmacogenomics Consortium (ICPC)

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Abstract

Rationale—The $P2Y_{12}$ receptor inhibitor clopidogrel is widely used in patients with acute coronary syndrome, percutaneous coronary intervention or ischemic stroke. Platelet inhibition by clopidogrel shows wide inter-patient variability and high on-treatment platelet reactivity is a risk factor for atherothrombotic events, particularly in high-risk populations. *CYP2C19* polymorphism plays an important role in this variability, but heritability estimates suggest that additional genetic variants remain unidentified. The aim of the International Clopidogrel Pharmacogenomics Consortium (ICPC) is to identify genetic determinants of clopidogrel pharmacodynamics and clinical response.

Study design—Based on the data published on www.clinicaltrials.gov, clopidogrel intervention studies containing genetic and platelet function data were identified for participation. Lead investigators were invited to share DNA samples, platelet function test results, patient characteristics and cardiovascular outcomes, to perform candidate gene and genome-wide association studies.

Results—In total, 17 study sites from 13 countries participate in the ICPC, contributing individual patient data from 8,829 patients. Available adenosine diphosphate (ADP) stimulated platelet function tests included Vasodilator-Stimulated Phosphoprotein (VASP) assay, Light Transmittance Aggregometry (LTA) and the VerifyNow $P2Y_{12}$ assay. A proof of principle analysis based on genotype data provided by each group showed a strong and consistent association between *CYP2C19**2 and platelet reactivity (p-value = 5.1×10^{-40}).

Conclusion—The ICPC aims to identify new loci influencing clopidogrel efficacy by using state-of-the-art genetic techniques in a large cohort of clopidogrel-treated patients in order to better understand the genetic basis of on-treatment response variability.

Keywords

clopidogrel; anti-platelet therapy; genome-wide association; CYP2C19; genotyping; candidate genes; pharmacogenetics; platelet reactivity; percutaneous coronary intervention; acute coronary syndrome; coronary artery disease

Background

In patients with coronary artery disease in which percutaneous coronary intervention (PCI) is performed, dual antiplatelet therapy with the cyclooxygenase-1 (COX-1) inhibitor aspirin and a $P2Y_{12}$ receptor inhibitor, commonly clopidogrel, is the recommended antiplatelet treatment to prevent recurrent atherothrombotic events, like stent thrombosis.[1, 2] Although the incidence of stent thrombosis is declining in recent years due to advances in clinical care, such as utilization of new stent designs, it remains a serious complication with a high mortality rate.[3] In addition, medical management with clopidogrel is effective in the prevention of recurrent cardiovascular events in patients with acute coronary syndrome (ACS), ischemic stroke and peripheral arterial disease.[4–6] However, substantial variability in on-clopidogrel platelet reactivity is well-documented. This variability leads to an increased risk for adverse thrombotic and bleeding complications in patients with high or low platelet reactivity, respectively.[7–9] Multiple factors influence variation in on-clopidogrel platelet reactivity, including genetic, anthropometric and clinical variables, as well as drug-drug interactions (e.g. calcium channel blockers, certain proton pump inhibitors such as omeprazole and esomeprazole, ketoconazole, morphine or St. John's Wort).[10–12] Smoking has been correlated to higher platelet inhibition by clopidogrel, but the data are controversial.[13, 14]

Conversion of clopidogrel into its active thiol metabolite by hepatic CYP P450 enzymes results in irreversible inhibition of platelet $P2Y_{12}$ receptors.[15] Polymorphisms in enzymes which are involved in the two conversion steps, in particular the *CYP2C19**2 (rs4244285) and *CYP2C19**3 (rs4986893) loss-of-function (LoF) alleles, result in lower clopidogrel active metabolite levels[16, 17], higher levels of on-treatment platelet reactivity[7, 17–23], and increased risk for on-treatment atherothrombotic events, in particular in the patients with the highest thrombotic risk.[24–29] The allele frequency of the *CYP2C19**2 polymorphism is ~15% in Caucasian populations and ~29–35% in Asian populations.[30, 31] These findings led the FDA in 2010 to add a boxed warning to the clopidogrel label, warning physicians that an

alternative antiplatelet drug should be considered in *CYP2C19**2 homozygote (poor metabolizer) patients.[32]

In addition to the *CYP2C19**2 and *3 polymorphisms, there are other genes in which polymorphism has been associated with impaired clopidogrel efficacy, such as *ABCB1, CYP2B6* and *PON1*. However, their role as determinants of clopidogrel response is controversial as the findings could not be reproduced in more recent studies.[33–37]

In contrast to factors related to increased atherothrombotic risk, the putative gain-of-function allele *CYP2C19**17 has been correlated with a higher clopidogrel active metabolite level and a reduction in on-treatment platelet reactivity.[38, 39] However, most of these reports have not taken into account that this allele is genetically linked to the *CYP2C19**2 allele, i.e., the allele containing the *17 polymorphism lacks the *2 polymorphism.[40] Also a rare decrease of function variant in carboxyesterase 1 (*CES1*), the enzyme responsible for converting clopidogrel into biologically inactive metabolites, has been reported to be associated with increased clopidogrel responsivity.[40] Recently, exome sequencing of patients with extreme pharmacodynamic responses to clopidogrel identified *B4GALT2* as a determinant of low on-treatment platelet reactivity.[41] Whether these variants result in better clopidogrel efficacy and/or increased bleeding risk remains to be determined.

Despite the relationship between *CYP2C19**2 and impaired platelet inhibition, as assessed by *ex vivo* adenosine diphosphate (ADP)-stimulated platelet aggregation, it only accounts for approximately 4–12% of the observed inter-individual variation in antiplatelet effect.[34, 42–44] Estimates suggest the heritability of the variability in response to clopidogrel to be as high as 70%, suggesting other genetic factors influencing clopidogrel efficacy.[44]

A previously conducted genome wide association study (GWAS) of clopidogrel response in 429 Amish subjects supported *CYP2C19**2 as the single major genetic determinant of clopidogrel response.[44] However, there were several additional common variants in or near other genes that showed nominal evidence of association with clopidogrel response in that investigation, but did not achieve genome-wide significance (p-value ~ 10^{-6}). If some of these variants represent true positive signals, they may be uncovered by larger sample sizes. To this end, the International Clopidogrel Pharmacogenomics Consortium (ICPC) was established to better define the genetic architecture of variable clopidogrel response. Participating study sites (N=17) contributed DNA samples, results of ADP-induced platelet function testing and clinical outcome data of patients treated with clopidogrel. A high quality genomic discovery resource was assembled consisting of DNA samples, pharmacodynamic data, major adverse cardiovascular outcomes, and relevant clinical characteristics.

This article describes the characteristics of the ICPC and provides a framework for the organization and execution of large pharmacogenetics studies.

Methods

Study design and population

An organizing committee of international pharmacogenomics investigators was established to define the scientific goals of the ICPC. These goals were to identify genetic variants that influence platelet aggregation and secondary clinical events in clopidogrel-treated subjects with the long-term objective of supporting the use of genetic information that would allow clinicians to make more informed treatment decisions for their patients requiring antiplatelet therapy.

The organizing committee invited 150 lead investigators of clopidogrel-related clinical studies registered in www.clinicaltrials.gov as of June 2011 to participate. Criteria for participation included availability of on-clopidogrel platelet reactivity data, availability of DNA samples for genetic analysis, and for secondary analysis, availability of clinical outcomes; however, studies did not have to fulfill all of these criteria. Eligible studies had to have a minimum of 50 planned enrollees. The phenotypic variables must have been obtained using predefined protocols and methods, and all patients had to be consented for genetic analysis and data sharing. All participating investigators signed a memorandum of understanding (included in the Supplemental Data section). Investigators at Stanford University (PharmGKB) serve as the centralized data coordinating center. Platelet function test results, demographic and cardiovascular outcome data, and other clinical data with a potential influence on platelet reactivity, such as age, sex, diabetes, body mass index, renal function and medication usage (i.e. calcium channel blockers and proton-pump inhibitors) were obtained from each study's investigators and curated. To date, 17 sites from 13 countries have joined the ICPC (Table I), contributing data representing 8,829 clopidogrel-exposed patients. The available sample size, including those with genotyping, and clinical outcome data, is described in Table II and Figure 1. DNA was available in 5119 ICPC subjects and sent to the University of Maryland where genetic analyses are coordinated. Cohort descriptions and the number of patients in which each platelet function test was performed are available in the online Supplemental Data section. The ICPC is supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Number U01HL105198. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

Study endpoints

The primary ICPC study endpoint is platelet function. Both platelet function measurements in patients on clopidogrel maintenance dose or after adequate loading dose were used, which was defined as at least 2 hours between a 600mg clopidogrel loading dose and platelet function testing, 6 hours after 300mg clopidogrel loading dose and 5 days after start of 75mg maintenance dose without extra loading dose. As platelet function in each study was measured using different platelet function tests, i.e. Light Transmittance Aggregometry (LTA), Multiplate Analyzer (Multiple Electrode Aggregometry; MEA), Vasodilator-Stimulated Phosphoprotein (VASP), and VerifyNow $P2Y_{12}$ assay, measurements were standardized across these different tests using a priority system laid out by the Phenotype

Subcommittee of the ICPC: VASP assay > VerifyNow $P2Y_{12}$ > ADP-induced LTA (higher ADP concentration > lower ADP concentration) > other tests. The platelet function test was then chosen from each site based on the highest ranking assay measured at that site that maximized the sample size. To standardize values across the different platelet function assays (all of which provide an assessment of ADP-stimulated platelet reactivity albeit expressed in different units), we standardized each measurement by subtracting out the mean platelet reactivity value and dividing by the standard deviation, thus expressing each measurement as a Z-score (i.e., the number of standard deviation units from the mean). These transformations were made within each site, thus allowing us to combine results by meta analysis despite the use of different platelet function assays. Standardized platelet function tests were available in 6270 ICPC subjects.

Secondary ICPC study endpoint included a composite clinical endpoint defined any of cardiovascular death, ischemic stroke, or spontaneous myocardial infarction. Stent thrombosis, defined by the Academic Research Consortium (ARC) criteria, was also ascertained but not considered as part of the composite clinical endpoint.[45] This rare but potentially lethal complication of stent placement will be analyzed separately. Major bleeding complications are captured in the database, although various definitions for bleeding were used in different study cohorts. The secondary endpoints were adjudicated locally using site-specific criteria. The ICPC includes a total of 5,819 subjects in which all components of the combined clinical endpoint are available. In those patients, 290 major adverse cardiac events (MACE) were observed (event rate 5.0%) during a mean (standard deviation [SD]) follow-up duration of 13 ± 9 months. The event rate is lower compared to the clopidogrel treated cohorts in the PLATO and TRITON studies, which might be explained by the higher percentage of ACS patients in those cohorts.[46, 47] In the subset of ICPC with DNA samples, 3426 patients have clinical data of whom 199 major adverse cardiac events were observed (event rate 5.8%) during a mean follow-up duration of 14 ± 11 months.

In addition to the main objectives of the ICPC, ICPC investigators as well as external investigators have the opportunity to access the combined data set for ancillary projects. Project proposals detailing research aims and analysis plans are reviewed and approved by the ICPC Steering Committee. Consortium members will not have access to personal identifiers. Consortium phenotype and genotype data will be deposited to public database (e.g. dbGAP) in accordance with NIH data sharing policies.

Genotyping

Genotyping will include both a candidate gene approach, in which a small number of genes and gene variants will be chosen based upon prior evidence for association with clopidogrel response, and an agnostic genome-wide approach using the Illumina Omni Express with Exome (OEE) chip, to identify novel loci associated with clopidogrel response. Data will be cleaned using the eMERGE QC pipeline.[48] eMERGE is the electronic MEdical Record and GEnomics Network, a National Human Genome Research Institute (NHGRI) funded consortium which has been combining clinical cohorts with biobanks linked to electronic health records. The genotype data cleaning process includes evaluation of sample and

marker call rate, gender mismatch, duplicate and HapMap concordance, batch effects, Hardy-Weinberg equilibrium, sample relatedness and population stratification. For GWAS, imputation to the 1000 Genomes reference dataset will be performed using the eMERGE Imputation Pipeline which includes SHAPEIT2 for phasing and IMPUTE2 for imputation.[49] eMERGE has developed a robust imputation and quality control pipeline for the combining of multiple datasets, which will be appropriate for the ICPC.

Database validation

To validate the ICPC central database, the association between cross-study harmonized platelet reactivity and *CYP2C19**2 was tested, as this association has been well-described in the literature.[7, 17–22, 50] All Caucasian patients with site-reported *CYP2C19**2 and harmonized ADP-induced platelet reactivity phenotype (n=5328) were selected. The analysis was adjusted for age and sex. All analyses were performed in SAS version 9.2 (SAS Institute Inc., Cary, North Carolina). Results confirmed that *CYP2C19**2 was independently associated with higher on-treatment platelet reactivity (Beta = 0.37; p-value = 5.1×10^{-40}).

Future directions

The ICPC is a collaborative platform for novel gene discovery related to clopidogrel effectiveness. It will serve as a well-powered resource to test new hypotheses and welcomes proposals from qualified investigators. ICPC will also serve as a replication cohort for genetic variants identified by other research groups worldwide. Proposals for new research questions can be send by email to the corresponding author of this paper.

Concluding remarks

With the introduction of the newer $P2Y_{12}$ receptor inhibitors prasugrel and ticagrelor, multiple antiplatelet drugs are now available for clinicians to use. Although prasugrel and ticagrelor are more effective in reducing atherothrombotic events in ACS patients, they increase the risk of bleeding compared to clopidogrel, and are more expensive.[46, 47] Given that alternative treatment options exist and pharmacodynamic and genetic testing options are available to predict or test the efficacy of clopidogrel, development of personalized antiplatelet strategies, to reduce atherothrombotic events and risk of bleeding, might significantly enhance patient care and potentially reduce costs; thus additional studies are warranted. Nevertheless, finding the optimal treatment strategy has proven to be difficult so far, as recent studies using different treatment modification strategies show contrasted results, owing in part to the different clinical settings and population vascular risk levels in the various studies.[51–57] Identification of novel gene variants that predict clopidogrel efficacy may provide important information to improve predictive algorithms for genotype-directed therapy. These studies also promise to provide new insights into platelet biology and to identify novel targets for more effective and safe antiplatelet therapy.

Summary

The aim of the ICPC is to find novel genetic markers which influence clopidogrel efficacy, using GWAS and candidate gene approaches combined with pharmacodynamic and clinical outcome data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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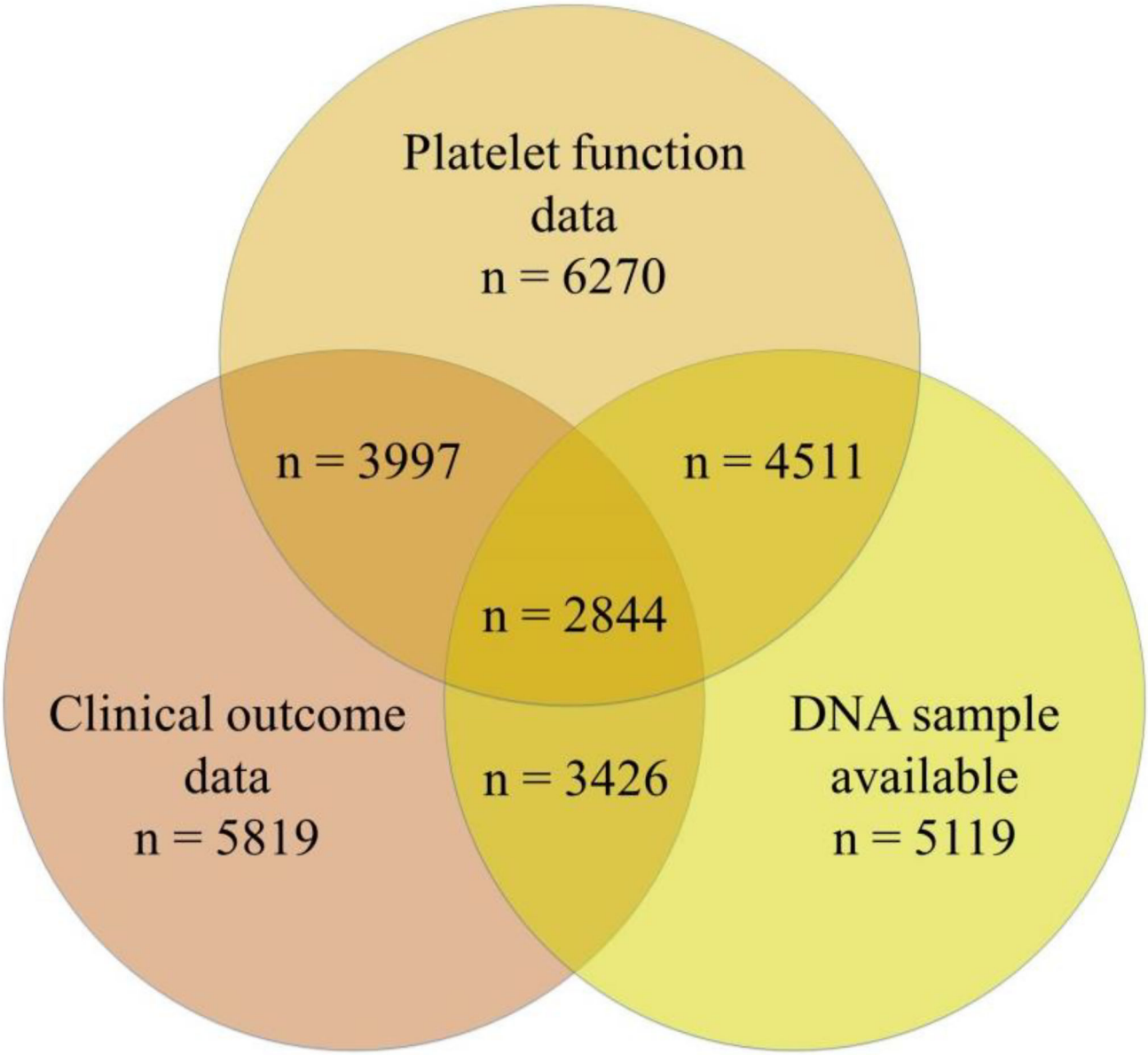

Figure 1.
Figure describing the subset of patients in which platelet function data, clinical outcome data and/or a DNA sample is available for analysis. Total number of patients is 8829.

Table I

Participating Study Sites

ICPC Primary Investigator	Country	No. patients
J.G. Shin	South Korea	2280
J.M. ten Berg	Netherlands	1024
D. Trenk	Germany	797
G.F. Gensini	Italy	736
J. Cleator, D. Roden	USA	693
A. Shuldiner	USA	687
P. Fontana, J. L. Reny	Switzerland, France	538
M. Schwab, T. Geisler	Germany	442
J.M. Siller-Matula	Austria	416
J. Déry	Canada	325
M. Valgimigli, G. Campo	Italy	234
D. Aradi	Hungary	192
M. Lee	Taiwan	160
L. Holmvang	Denmark	106
P. Gurbel	USA	79
I. Fernández-Cadenas	Spain	70
D. Alexopoulos	Greece	50
PharmGKB		
T. Klein	USA	
L. Gong	USA	
P-STAR		
M.D. Ritchie	USA	

Table II

Baseline Characteristics of Study Participants

	Total cohort N=8829	**Anticipated genotyped cohort* N=4511**
Self-reported race		
White	70.8	94.6
Asian	27.9	4.9
Other	1.3	0.5
Gender (male)	71.2	76.4
Age (years)	62.8 ± 13.0	63.5 ± 12.5
Body Mass Index (kg/m^2)	26.9 ± 4.7	27.4 ± 4.4
Diabetes	26.0	23.3
Current smoker	19.5	22.3
Hypercholesterolemia	53.8	66.1
LVEF <35%	5.2	6.1
Aspirin use	94.7	93.2
Statin use	75.0	82.3
CYP2C19 *2 carrier (site-reported)	35.7	31.9
CYP2C19 *17 carrier (site-reported)	28.6	36.2
Coronary artery disease (indication for clopidogrel use)	81.4	94.3
- acute coronary syndrome	33.7	44.2
- PCI performed	92.5	87.6
- for acute coronary syndrome	36.4	56.6
- for non-urgent PCI	63.6	43.4

Data are expressed in % or Mean ± SD

*All patients with both a DNA sample and platelet function test result available

Abbreviations: LVEF = left ventricular ejection fraction, PCI = percutaneous coronary intervention, SD = standard deviation