CES1 Carriers in the PAPI Study
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STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

The purpose of this investigation is to assess the impact of genetic variation on response to clopidogrel as well as dual antiplatelet therapy (i.e. clopidogrel and aspirin), as assessed by ex vivo platelet aggregometry, in healthy Amish individuals. The research design of this investigation closely mirrors the study design implemented in the Pharmacogenomics of Anti-Platelet Intervention (PAPI) Study (NCT00799396). The primary reason for the significant overlap in study design is that it is our intention to integrate data collected in this protocol with currently existing data generated as part of the PAPI study in order to perform robust, well-powered statistical analyses pertaining to clopidogrel and dual antiplatelet therapy response.

Specifically, we enrolled 6 healthy Amish participants who were identified using genomic and bioinformatic approaches. Enrolled participants underwent a two-stage intervention with clopidogrel (300 mg loading dose then 75 mg per day for the next 6 days), followed by clopidogrel (75 mg) plus aspirin 324 mg (4 baby aspirin) for 1 day. Platelet aggregation studies were performed before and after each intervention.

Home visit to screen for eligibility: A research nurse and an Amish community liaison visited potential research subjects in their homes. This mechanism is culturally appropriate since the Amish do not have phones in their homes and prefer face-to-face contact to letters. The nurse explained the study and, if the individual was interested in participating, obtained informed consent and HIPAA authorization. Following informed consent, vital signs as well as medical and family history will be obtained. Approximately 10 mL of blood were drawn for CBC, HbA1c, comprehensive metabolic panel, and thyroid function test (TSH) to evaluate eligibility. Exclusion criteria were identical to the original PAPI Study and can be elsewhere in this submission. Briefly, participants were excluded if they were pregnant, currently breastfeeding, had a history of a bleeding disorder or major spontaneous bleed, hypertensive, creatinine levels > 2.0 mg/dL, ALT or AST > 2 x the upper limit of normal, hematocrit < 28%, TSH < 0.4 or >5.5 mIU/L, platelet count > 500,000 or < 75,000, currently taking antiplatelet or anti-coagulant medication, history of cardiovascular disease or diabetes, taking vitamins or other supplements and unwilling or cannot safely, in the opinion of the study physician, discontinue their use at least 1 week prior to protocol initiation.

Subjects were informed that to participate in this study, they had to discontinue for a period starting 7 days prior to the initial clinic visit any vitamins, supplements and medications that might affect the results of the study. Specifically, if a subject is on medications that may potentially interfere with the planned intervention, he/she was instructed to consult with his/her physician regarding discontinuing such therapy prior to study enrollment. Alternatively, with the subject's permission, the study physician contacted the subject's physician to discuss and obtain permission for medication withdrawal. If in the opinion of the subject's physician and the study physician, discontinuation of medications for the duration of the study did not pose any undue health risks, medications were discontinued one week before the first clinic visit. If a medication required tapering, this commenced two weeks prior to the first clinic visit. Similarly, all vitamins (except daily multivitamins) and other supplements were withdrawn for 7 days prior to the first clinic visit. If the research subject was on anti-platelet medications, these agents were withdrawn 14 days prior to the first clinic visit.

Female research participants were informed that they could not participate in this study if they are pregnant. All female subjects had an assessment of child-bearing potential. Pregnancy status was determined by self-report at the screening visit. A urine pregnancy test was performed at the clinic visit #1 prior to drug administration.

Following review of screening information and lab results, eligibility was confirmed. Eligible participants completed studies during the course of two visits to the Amish Research Clinic in Lancaster, PA.

Clinic Visit #1 (Baseline Platelet Function): Research subjects were transported to the Amish Research Clinic after an overnight fast where heights, weights, and vital signs will be measured. Women of child bearing age underwent a pregnancy test. Fasting blood samples were obtained to assess circulating lipid levels (LDL, HDL, and triglycerides), evaluate baseline measures of agonist-stimulated platelet aggregation, and for sample banking. Participants were observed while taking the loading dose of clopidogrel (300 mg), discharged to home with additional tablets, and instructed to take 75 mg/ day for the following 6 days at the same time each day. Drug adherence was assessed by participant logbook and pill count performed by a study nurse at the beginning of Clinic Visit #2.

Clinic Visit #2 (On-Drug Platelet Function): On the 7th day, subjects returned to the clinic. A pill count and logbook check were performed to assess adherence. Fasting blood samples were drawn approximately 2 hours post-dose for measurement of agonist-stimulated platelet aggregation and sample banking. Visit 2 may have occurred up to 10 days after Visit 1 in which case the drug was taken for 1 to 3 additional days. After the participants completed all of the procedures pertaining to on-clopidogrel platelet function, they received a one-time dose of 324 mg of aspirin (oral ingestion of 4 baby aspirin tablets). Approximately 2 hours after the participants took the aspirin, repeat measures of platelet aggregation were recorded and blood samples were banked.

Assessment of Platelet Function: At both clinic visits, platelet-rich plasma (PRP) was isolated from fasting blood samples drawn into 3.2% citrate-anticoagulated tubes (Becton-Dickinson, Franklin Lakes, NJ) and platelet counts were adjusted to approximately 200,000 platelets/μl using platelet-poor plasma. Platelet function was assessed by optical aggregometry using a PAP8E Aggregometer (Bio/Data Corporation, Horsham, PA) according to the manufacturer's instructions after stimulation with collagen (5 μg/ml) and ADP (20 μM) and was expressed as the maximal percentage change in light transmittance using platelet-poor plasma as a referent. Platelet function was assessed twice during Clinic Visit #2; before and after administration of aspirin as described above.

Distributions of summary statistics (e.g. age, sex, change in platelet function) were generated using SAS 9.2 (Cary, NC). Change in clopidogrel-induced platelet aggregation in response to ADP and collagen were calculated by subtracting observed post-clopidogrel maximal platelet aggregation values from baseline (i.e. pre-drug) values of the same platelet agonist. Similarly, change in dual antiplatelet therapy (DAPT, clopidogrel and aspirin)-induced platelet aggregation in response to ADP and collagen were calculated by subtracting observed DAPT maximal platelet aggregation values from baseline (i.e. pre-drug) values of the same platelet agonist.