1. Full Title:

Association of platelet GP Ibα Polymorphisms and p-selectin glycoprotein LIG and -1 (PSGL-1) polymorphism with CHD

1b. Abbreviated Title (Length 26):

Platelet GP Ibα and p-selectin polymorphisms

2. Writing Group (list individual with lead responsibility first):

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3. Timeline:

For the analysis of the GP Ibα genotypes, as well as P-Selectin glycoprotein ligand-1 genotype, DNA samples from the reduced random cohort sample and the incident CHD cases will be needed. We expect to complete the analysis within two months. A report formatted as a draft manuscript can be presented to the Steering Committee three months after the completion of the analysis.

4. Rationale:
Platelets play an essential role in the development of arterial thrombi, which can lead to myocardial infarction and stroke. In the development of the coronary thrombus, platelets can become activated initially by two means: by the exposure of thrombogenic substances in the artery wall following plaque rupture and, by the high shear forces found in regions of arterial stenosis. One platelet receptor that participates in both of these processes is the glycoprotein Ib-IX-V complex, which is the first receptor to attach the platelets to the vessel wall by recognizing von Willebrand factor exposed at the site of vessel damage. The interaction of the complex with soluble von Willebrand factor in regions of high shear is also a sine qua non of shear-induced platelet aggregation. Thus, polymorphisms of this receptor complex are likely to be determinants of thrombotic risk. Those variants that lower the threshold for thrombosis may represent risk factors for myocardial infarction or stroke.

One polymorphism of GP Ibα that affects its ligand-binding region involves the presence of threonine or methionine at amino acid residue 145 (substitution of T for C at position 434 of the coding region of GP Ibα gene). This polymorphism affects the conformation of the vWF-binding region of GP Ibα, as evidenced by alloantibodies. Heterozygous fetuses of homozygous mothers are at risk of developing thrombocytopenia. This polymorphism is the basis of the KO (Sib or HPA-2) alloantigen system. Recently, we have found that the different variants display a differential reactivity with monoclonal antibodies that block vWF binding to the region (unpublished data). The frequency of the less common allele for KO is about 10% in all tested populations.

We have characterized another novel polymorphism in the GP Ibα gene (the ligand-binding subunit of the complex) that changes the surface density of this protein on platelets. This polymorphism alters one base pair in the 5′ untranslated region of the GP Ibα gene in the vicinity of the initiation codon (nucleotide #3064, 5bp upstream from ATG start codon). This region is important in the efficient translation of the mRNA. As a result, individuals with different polymorphic variants of the GP Ibα gene express significantly different amounts of this protein on their platelets. We propose to study the prevalence of this Kozak polymorphism in a defined subset of a large population for which a number of clinical and laboratory parameters have been defined in a prospective study design. The frequency of the less common allele is about 15%.

PSGL-1 is the only high-affinity molecule on the surface of intact leukocytes for endothelial or platelet P-selectin. The interaction between PSGL-1 and endothelial P-selectin is a prerequisite for adhesion of the leukocytes to the endothelial cells involving the (ICAM-1). PSGL-1 is a transmembrane protein that contains numerous tandem repeats of ten amino acids within a mucin-like domain in its extracellular domain. These variable numbers of tandem repeats (VNTRs) may affect receptor function and the inflammatory process. The PSGL-1 polymorphism has three alleles with the following approximate frequencies: A (65-80%), B (15-35%) and C (about 2%).

5. Main Hypotheses:
Since the polymorphism of GP Ibα affects the number of the adhesion receptors on the surface of the platelets, it may also influence the platelet adhesion capability and the chance of thrombotic events. ARIC database will allow us to see if this significance in the number of receptors translates into a significant risk of thrombotic events.

Also, variable number of tandem repeats in PSGL-1 may influence the leukocyte adhesion (inflammation) as a step in the process of atherosclerosis before arterial thrombosis occurs. We propose to investigate the association between level of P-selectin (data already exist in ARIC data base) and PGHS-1 polymorphism.

6. **Data (variables, time window, source, inclusions/exclusions):**

We will use DNA samples from individuals belonging to the random cohort and incident CHD events to determine their GP Ibα genotype as it relates to the KO, Kozak sequence, and PSGL-1 polymorphisms. These data will be analyzed locally by Chul Ahn and then transferred to the CCSS for data verification.

**References:**


4) Afshar-Kharaghan V, Khoshnevis-Asl M, Lopez JA. Kozak sequence polymorphism is a major determinant of surface level of platelet adhesion receptor. (Submitted)

5) McEver RP, Cummings RD. Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest* 1997; 100:S97-103