1. Full Title: Correlation of platelet membrane $\alpha_2\beta_1$ gene polymorphism with incident myocardial infarction (IMI)
   b. Abbreviated title: platelet membrane $\alpha_2\beta_1$ gene polymorphism and IMI

2. Writing Group:

   Lead: Robert Sweetman
   Address: Children's Hospital of Orange County
   Division of Hematology/Oncology
   455 South Main Street
   Orange, CA 9286
   Phone: (714) 532-8636
   Fax: (714) 532-8883
   E-mail: rseetman@hotmail.com

   Diane Nugent (djnugent@earthlink.net)
   Thomas Kunicki
   Nena Aleksic
   Kenneth Wu
   Eric Boerwinkle
   Ed Davis

3. Timeline:

   For the analysis of the polymorphisms of the $\alpha_2$ gene DNA samples from the so-called "2 group" random cohort sample and the incident CHD cases will be needed. Dr Sweetman will be doing the majority of sample analysis for the $\alpha_2\beta_1$ expression. It will be his highest priority and he will have the majority of his time committed to this project between now and July 1997. In July, Dr. Sweetman will complete his fellowship and will be accepting academic appointment where he will has significant clinical responsibilities. However he still anticipates having the time to complete the project.

4. Rationale:

   Specific polymorphisms of the $\alpha_2$ gene are associated with markedly increased number of $\alpha_2\beta_1$ collagen receptors on the surface of platelets. Furthermore the level of platelet $\alpha_2\beta_1$ has been shown to be an inherited trait. The desired segment of the $\alpha_2$ gene is amplified using leukocyte DNA as a template. PCR is performed using primer pair GTGTTTAACCTTGAACACATATAAAAACC (bp 715-741) plus
CTCAGTATATTGTCATGGTGCTTG (bp 940-965) in the first stage, then the nested primer pair TCCCtcgAgTCCCAATATGGTGGGACCTCAC (XhoI: bp: 775-797) plus CTCtCTAgATTGTCATGGCATTGATCAATCAC (bp 931-956; XbaI) in the second stages. The product is a ~4.5 Kb DNA segment that includes both the 807 and 873 allelic sequences and 4.35 Kb of intervening, noncoding sequence. It likely represents a single large intron separating two exons that encode each of the allelic sequences. The \textit{XhoI} and the \textit{XbaI} restriction sites were introduced to facilitate subcloning of the segment. Upon digestion with \textit{XbaI}, it became apparent that there is one additional restriction site within the noncoding sequence. Thus, complete digestion with \textit{XhoI} and \textit{XbaI} yields a 3.7 Kb 5' fragment and a 650 bp 3' fragment. The sequence of the entire 650 bp fragment as well as 600 bp of the larger fragment were obtained by dideoxy method using Sequenase 2.0. Using the newly formed intron sequence, PCR conditions were established to amplify smaller DNA fragments that would include either the 807 or the 873 allelic sequences. The 298 bp genomic fragment that includes the 807 allele is amplified. The allelic sequences were originally detected by hybridization to the probes for 807C (associated with low receptor expression) or 807T (associated with high receptor expression) as well as an oligonucleotide sequence corresponding to the intron serving as the positive control. Similarly a 398 bp genomic fragment that includes the 873 alleles is amplified and the allelic sequences at 873 were detected to hybridization to probes 873G or 873A. There was direct correlation between the two alleles so that 807C is always associated with 873G and 807T is always associated with 873A. We currently test samples only for the 807 allele. Kunicki and his lab recently demonstrated that there exists a unique bgI II site in the 807T allele that is not present in the 807C site. Therefore by using a different PCR reaction and a bgI II digest eliminates the need for hybridization. There has been 100% correlation with samples analyzed both ways.

5. Main Hypothesis:

We postulate that patients with thrombotic disorders will exhibit increased expression of the $\alpha_2\beta_1$ gene Polymorphisms that are associated with increased receptor density and thus an inherited risk towards thrombotic disorders.

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

Data will be sent to the CC for the analysis.