

## 1996 MINUTES

### PREDICTIVE VALUES OF HEMOSTATIC VARIABLES IN VASCULAR DISEASE SUBCOMMITTEE

Sunday 23, June, 1996, 13.00 - 17.00

Room Safir, Fira Palace Hotel

Barcelona, Spain

Chair: Gordon D. O. Lowe, UK

Co-Chair: Y. Stirling, UK; K. Bauer, USA; M. Hultin, USA; R. Hull, Canada

Dr. Lowe had been asked to continue as acting chairman during 1996, and had asked Dr. Stirling to continue as co-chairman for this meeting in the absence of other co-chairmen.

Overview of Predictive Value of Hemostatic Variables in Vascular Disease: Dr. Lowe reported that this was almost complete and would be circulated soon within the subcommittee prior to submission to SSC. Fibrinogen: Dr. Lowe reported results from the Scottish Heart Health Study confirming the predictive value of fibrinogen (Clauss assay) for CHD events in men and women, especially fatal events. Using the international standard, the upper tertile (in which the relative risks was about 2.0) was defined as about 3.0 g/L. Dr. Lowe reported that in the inter-laboratory comparison of Clauss fibrinogen, published variation appeared largely attributable to variations in standards. Dr. S. Kitchen reported continuing wide variation between "routine" laboratories for "normal" fibrinogen assays in UK-NEQAS, although this may be decreasing with increasing use of the International Standard.

Factor VII: Dr. Stirling reported the results of a 20-centre calibration exercise for the SSC standard. A potency of 1.02 units/ampoule by factor VIIc assays was accepted. Data for chromogenic, ELISA and factor VIIa assays was encouraging but insufficient for formal analyses. A report would be prepared for publication. Dr. Kitchen reported good agreement between laboratories for factor VIIc assays in UK-NEQAS. Dr. Stirling suggested preparation of guidelines on factor VIIc assays in epidemiological studies and assay of the SSC standard by the Northwick Park assay. Dr. Kluff suggested comparison of factor VII assays in "high risk" samples.

Factor VIII/von Willebrand Factor: Dr. I. Jennings reported wide variation between laboratories for factor VIIIc and vWF antigen assays in UK-NEQAS. Variation in reference plasmas appeared most important, but variation in substrates and deficient plasmas also had an effect.

Fibrinolysis: Dr. C. Kluff reported the current standardization program of the Fibrinolysis Subcommittee, which was an integrated approach to standards and methods.

Coagulation Inhibitors: Dr. I. Walker reported the design and preliminary results of the EPCOT Study, a prospective case-control study of thrombotic risk in persons with congenital deficiencies in coagulation inhibitors. Drs. F. Rodeghiero and G. Lowe reported distributions and associations of antithrombin, protein C, protein S and heparin cofactor II in two large population studies.

Reference ranges could not be defined parametrically, and varied with age, sex, and hormonal, lipid and lifestyle factors. Dr. I. Jennings reported wide variation for antithrombin, protein C, protein S, and APC resistance between laboratories for both assays and diagnosis in UK-NEQAS. Assay methods, source of kits, reference plasmas, expression of results and definition of reference range were each contributory.

Gene Polymorphisms: Dr. L. Iacovello reviewed gene polymorphisms related to haemostatic factors and risk of arterial vascular disease. An ETRO Working Group has been established to study the relationships to geographic variation in CHD risk across Europe. Dr. Iacovello proposed a register of such studies. Dr. N. Sala proposed a new nomenclature for existing and future gene polymorphisms.

## 1997 MINUTES

### PREDICTIVE VARIABLES AND CARDIOVASCULAR DISEASE SUBCOMMITTEE

Friday, 6 June, 1997, 13:00-17:00

Tiziano, Fortezza da Basso

Florence, Italy

Chair: K. Bauer, USA

Co-Chairs: R. Hull, Canada; S. Humphries, UK; G. Lowe, UK

The number of people attending this subcommittee meeting was estimated at 450.

**Epidemiologic studies:** Dr. George Miller led off the session by presenting an overview of three major prospective epidemiologic studies. These include the ARIC (USA), PROCAM (Münster, Germany) and Second Northwick Park Heart ( NPHS II) Studies (UK). Data were presented from NPHS II indicating a steep decline in coronary event rates, which has been greater than anticipated at its inception in 1989. As compared to NPHS I which was initiated in the 1970s, NPHS II includes fewer current smokers (26% versus 46%) and participants had lower mean diastolic blood pressures at entry. Plasma fibrinogen measurements were also significantly reduced in NPHS II as compared to NPHS I.

**Genetic Polymorphisms:** Dr. L. Iacoviello presented an update from the ETRO working party on "Population Genetics of Hemostatic Risk Factors for Arterial Vascular Disease." She discussed a meta-analysis of studies examining the role of the 4G/5G polymorphism in the PAI-1 promoter as a risk factor for myocardial infarction. The 4G/4G allele was associated with a 1.24-fold increased risk of coronary heart disease (CHD).

Dr. F. Rosendaal reviewed data from the Leiden Thrombophilia Study that the G20210A mutation in the prothrombin gene is a risk factor for venous thrombosis. This mutation is correlated with elevated prothrombin levels. Dr. Michael Laffan (UK) discussed data from a thrombophilia clinic population showing that elevated factor VIII:C is a risk factor for venous thrombosis. These patients also had elevated VIII:Ag levels which correlated with VIII:C. Thus far, it has not been possible to define genetic abnormalities that are associated with these elevations.

**Hyperhomocysteinemia:** Dr. M. Cattaneo (Milan) presented an overview of hyperhomocysteinemia as a risk factor for venous thrombosis. He reviewed methodologic issues related to plasma homocysteine measurements including performance of assays post-methionine loading. Dr. A. Tripodi (Milan) presented plans for a multi-center study of plasma homocysteine assays. Dr. Armando D'Angelo discussed his center's experience in evaluating hyperhomocysteinemic patients.

**Fibrinogen:** Dr. Lowe (UK) reported results from several Scottish epidemiologic studies confirming the predictive value of fibrinogen for CHD events in men and women. He commented that variability of fibrinogen measurements has decreased with use of the

international fibrinogen standard. Also improved predictive power of fibrinogen measurements for CHD can be obtained using a heat nephelometry assay.

Dr. F. Haverkate (Netherlands) briefly presented results of antithrombin III, protein C, protein S, and APC resistance determinations performed by the ECAT foundation in 70 laboratories.

**Parameters of Hemostatic Activation:** Dr. J. Morrissey (USA) described a clot-based assay for measuring free factor VIIa levels in plasma and the results of some clinical investigations using this method. He also presented preliminary data regarding potential explanations for a ten-fold difference in normal levels between the clot-based assay and an immunoenzymatic assay reported by Philippou and Lane.

**Inflammation and Hemostatic Variables:** Dr. R. Tracy (USA) and F. Haverkate reviewed the topic of inflammation as it relates to hemostatic variables and CHD risk. Recent data were presented regarding elevated levels of C-reactive protein as a coronary risk factor.

**1998 MINUTES**  
**PREDICTIVE VARIABLES AND CARDIOVASCULAR DISEASE SUBCOMMITTEE**  
**Monday, 22 June, 1998, 8:00-12:00**  
**Cankerjev Dom**  
**Ljubljana, Slovenia**  
**Chair: K. Bauer, USA**  
**Co-Chairs: R. Hull, Canada; G. Lowe, UK;**  
**F. R. Rosendaal, The Netherlands**

The number of people attending this subcommittee meeting was estimated at 75.

**Hyperhomocysteinemia.**

Dr. M. Cattaneo discussed the topic of hyperhomocysteinemia with special emphasis on methodologic issues. He first presented data regarding comparing the two- and four-hour methionine loading tests for the diagnosis of hyperhomocysteinemia. Based on results in 371 patients, he concluded that there was good concordance between the two tests. However they were not equivalent in that the four-hour test was more sensitive to methionine intolerance. It was therefore suggested that the two hour protocol should not replace the four hour protocol. He next presented data from a study of 628 healthy controls which determined that the methionine loading test does not only explore the trans-sulfuration pathway in homocysteine metabolism. Finally, he discussed results in 200 healthy controls and 52 patients with previous thrombotic episodes comparing an ELISA assay to standard HPLC methodology for plasma total homocysteine measurements. A good correlation was found for total homocysteine concentrations less than 30-35 micromolar; thus the diagnostic accuracy of the ELISA assay was considered excellent for the diagnosis of fasting hyperhomocysteinemia and good for methionine loading tests.

**Genetic polymorphisms.**

Dr. L. Iacoviello presented an update from the recent ETRO working party meeting on "Population Genetics of Hemostatic Risk Factors for Arterial Disease." She discussed meta-analyses of studies examining the role of the 4G/5G polymorphism in the PAI-1 promoter, the Bcl 1 polymorphism in the beta-fibrinogen gene, the repeat polymorphism in the tissue plasminogen activator gene, and the PLAI polymorphism in the platelet glycoprotein IIIA as risk factors for myocardial infarction. Plans will be made to formalize interactions between the ETRO working party and the activities of this subcommittee.

**Overview of Predictive Variables.**

Dr. G. Lowe reviewed data from a recently published metaanalysis of fibrinogen as a risk factor for ischemic heart disease (Danesh et al., *JAMA* May 1998) indicating a two-fold increased risk with a tighter confidence interval in patients with fibrinogen levels in the top tertile. He reviewed the various methodologies for fibrinogen assays and emphasized the need for attention to this issue in the interpretation of the metaanalyses. He next presented results of a study in 1,661 healthy subjects (aged 25-74) comparing results of fibrinogen assays performed by Clauss assay, a prothrombin time-derived method, and immunonephelometry. Correlations between the three assays ranged between 0.69 and 0.85. Dr. Lowe is preparing an overview article on the topic of predictive variables and cardiovascular disease for submission by this subcommittee for submission to *Thrombosis and Haemostasis*.

**Factor VII Polymorphisms.**

Dr. L. Iacoviello presented her results on factor VII polymorphisms as a risk factor for ischemic heart disease in patients with a positive family history and young patients. Potential reasons for different results (i.e., patient selection, ethnic differences in polymorphism frequency, as well as environmental factors) were discussed. Further discussion of this topic will be presented at the SSC meeting in Washington next year along with discussion of other genetic risk factors including factor V Leiden, prothrombin 20210A, and factor XIII-Val34Leu polymorphisms.

## **1999 MINUTES**

### **PREDICTIVE VARIABLES AND CARDIOVASCULAR DISEASE**

**Saturday, 14 August 1999**

**1:00 to 5:00 PM**

**Room 30/31**

**Washington Convention Center**

**Washington, DC**

**Chair: K. Bauer, USA**

**Co-Chairs: R. Hull, Canada; L. Iacoviello, Italy; G.D.O. Lowe, UK**

The number of people attending this subcommittee meeting was estimated at 200.

Fibrinogen Assays. Dr. Ian Mackie, chair of the British Society of Haematology Working Party on Fibrinogen Assays, reported on a comprehensive evaluation of commercial fibrinogen standards, Clauss fibrinogen assays, and prothrombin-derived methods. These studies were performed on both photo-optical and mechanical coagulation analyzers at several sites in the U.K. In addition to lyophilised plasmas, a large number of plasma samples were analysed from patients with disseminated intravascular coagulation, liver disease, dysfibrinogenaemia, as well as those with elevated fibrinogen levels. The major findings included apparent errors of calibration in some commercial fibrinogen reference preparations, differences in results between Clauss kits, and a variety of discrepancies in prothrombin-derived assays, which were dependent on the thromboplastin and standard preparations used.

Overview of the Results of the Second Northwick Park Heart Study. Dr. George Miller presented an overview of the results of this prospective cardiovascular survey designed to prospectively look for associations between coagulation activation markers and a first episode of myocardial infarction in middle-aged males.

Meta-Analysis of Haemostatic Variables in Prediction of Cardiovascular Disease. Dr. John Danesh presented the results of meta-analyses for fibrinogen, C-reactive protein, albumin, and leucocyte count as markers of coronary risk. Using studies published before 1998 that included 4,000 cases of myocardial infarction, a 1 gram/L increase in fibrinogen was associated with a 1.8-fold increased risk. The analysis of C-reactive protein included 1,053 cases and conferred a 1.7-fold increased risk. Among 3,770 cases of coronary heart disease, the population in the bottom third of the population with respect to serum albumin level had an increased risk as compared to the top third. Among 8,054 cases, the population in the top third of the population for hematocrit had a 1.3-fold increased risk as compared to the bottom third. Data was also presented for plasma viscosity and erythrocyte sedimentation rate.

Overview of Committee Report on "Predictive Variables and Cardiovascular Disease." Dr. Gordon Lowe presented an overview of an article on this topic that is being prepared for submission to *Thrombosis and Haemostasis*. The variables to be included include Factor VII, tPA, PAI-1, coagulation inhibitors, and fibrinogen. The preparation of this paper has been delayed awaiting more prospective studies and comparative studies of different assays. It is anticipated that a draft manuscript will be circulated by the end of 1999 and hopefully ready for SSC approval at next year's Annual Business Meeting in Maastricht.

The Genetics of Factor XIII, Fibrinogen, and Platelet Glycoproteins and Vascular Disorders. Drs. Peter Grant and Rashta Anwar presented data regarding the protective role of the Factor XIII-Val34Leu polymorphism for arterial as well as venous thrombosis. Data was presented indicating that the activation peptide of Factor XIII is actually released by a lower concentration of thrombin for the mutant Factor XIII molecule as contrasted with the wild type molecule. Dr. Angela Carter presented data regarding fibrinogen polymorphisms and the PI<sup>A2</sup> polymorphism in the platelet glycoprotein IIIA gene as risk factors for myocardial infarction. Dr. Pascal-Goldschmidt also presented data regarding the role of the PI<sup>A2</sup> polymorphism as a risk factor for myocardial infarction.

Report of the ETRO Working Party on "Population Genetics of Hemostatic Risk Factors for Arterial Vascular Disease." Meta-Analysis of Genetic Polymorphisms and the Risk of Myocardial Infarction in the Young. Dr. Licia Iacoviello presented an update from this group which included a meta-analysis of the PI<sup>A2</sup> allele. Among 9,274 cases and 14,675 healthy controls, the polymorphism had a weak effect on the risk of coronary artery disease. The effect however was double in subjects younger than age 60 (odds ratio=1.22) and strongest in restenosis after angioplasty (odds ratio=1.31).

Overview of Activated Protein C Resistance (APCR) and Factor V Leiden in Prediction of Thrombosis. Drs. Gordon Lowe and M. McColl presented data on the role of APCR due to the Factor V-Arg506Gln mutation in venous thrombosis. Recommendations were presented regarding populations warranting screening and the implications of a positive diagnosis with respect to patient management. Dr. Lowe presented a meta-analysis of the role of APCR in deep venous thrombosis following total hip replacement. He concluded that APCR is probably associated with an increased risk of both asymptomatic (venographic) deep venous thrombosis and confirmed clinical thromboembolism after elective total hip arthroplasty despite routine antithrombotic prophylaxis.

## **PREDICTIVE VARIABLES IN CARDIOVASCULAR DISEASE**

**16 June 2000**

**13:30 to 17:3**

**Room 0.4**

**Maastricht Meeting and Convention Center**

**Chairman: K.A. Bauer--USA**

**Co-chairmen: M. Cushman--USA; P.J. Grant--UK; R. Hull--Canada;  
L. Iacoviello--Italy; G.D.O. LoweUK**

The number of people attending this subcommittee meeting was approximately 50.

Homocysteine. Dr. A. Tripodi presented results of a collaborative study evaluating different methods (by HPLC, enzyme immunoassay ó EIA, and fluorescence polarization immunoassay ó FPIA) for measuring homocysteine levels. The conclusions of the study were that the FPIA method gave a lower coefficient of variation than the other methods and that the performance characteristics of the FPIA and HPLC methods compare favorably to one another. The comparability of standards is a problem and it was felt that establishment of a plasmatic standard would be helpful in the standardization of assay methodologies. Dr. M. Cattaneo presented data showing that high post-methionine load total homocysteine levels and low plasma vitamin B6 levels are independently associated with an elevated risk for deep venous thrombosis. Low folic acid levels were less strongly associated with risk.

Meta-Analysis of Haemostatic Variables in Coronary Heart Disease. Dr. J. Danesh presented the results of meta-analyses for fibrinogen, the G/A ó455 beta-fibrinogen gene polymorphism, fibrin D-dimer, von Willebrand factor, lipoprotein (a), and C-reactive protein as markers of coronary risk. Using the British Regional Heart Study as well as many other published studies, data was presented for incident disease associations in apparently healthy populations as well as for disease versus undiseased cohorts. All of the aforementioned variables with the exception of the beta-fibrinogen gene polymorphism were found to be significantly associated with coronary heart disease.

Effect of Factor XIII-Val34Leu on Fibrin Structure and Function. Dr. R. Ariens presented data showing that the catalytic efficiency of thrombin-mediated factor XIII activation is doubled by the presence of the Val34Leu polymorphism as compared to wild type. Early covalent cross-linking of factor XIII-Val34Leu produces more gamma-gamma and alpha-alpha crosslinks, thereby inhibiting lateral aggregation processes. This leads to formation of a fibrin clot with a finer structure, thinner fibers, and smaller pores as compared to fibrin formed using wild type factor XIII.

Role of Hemostatic Gene Polymorphisms in Venous and Arterial Thrombotic Disease. Dr. P. J. Grant gave an overview of a large amount of information on this topic that was recently published by he and Dr. D. Lane in BLOOD. While clear associations between genetic polymorphisms, intermediate phenotypes, and disease are found for deep venous thrombosis

(e.g., factor V Leiden leads to resistance to activated protein C), this has frequently not been the case for polymorphisms reported to be associated with arterial thrombotic disease. The results of different studies seeking associations between hemostatic gene polymorphisms and arterial thrombotic disease have largely given inconsistent results with respect to the predictive value of most genetic variants. A discussion ensued regarding future prospective studies in this field and the ultimate utility of such data given the data to date.

Emerging Candidate Genes for Cardiovascular Disease. Dr. L. Iacoviello presented new data that a 6511 C to T polymorphism in the IL1-beta gene is independently associated with an increased risk of myocardial infarction in a young Italian population with a positive familial history. The monocytes of patients homozygous for the CC polymorphism exhibited increased release of IL1-beta after 24 hours of stimulation by lipopolysaccharide. Data was also presented on the role of various factor VII gene polymorphisms (Arg353Gln, decanucleotide insert, and 6402 G to A) and CHD risk in this population. The Arg353Gln polymorphism and the decanucleotide insert are in strong linkage disequilibrium. Arg353Gln has been reported to be protective against coronary heart disease by Dr. Iacoviello. It was pointed out that the allele frequencies for these two polymorphisms are significantly different between Italian and Northern European populations, thereby providing a potential explanation for the very different results that have been reported. Data was also presented that the 6402 G to A polymorphism was not associated with an increased risk for premature myocardial infarction.

## **PREDICTIVE HAEMOSTATIC VARIABLES IN CARDIOVASCULAR DISEASE**

**6 July 2001  
13:00 to 17:00  
Room 242  
Palais des Congrès**

Chairman: L. Iacoviello--Italy

Co-chairmen: K.A. Bauer--USA; M. Cushman--USA; P.J. Grant--UK; R. Hull--Canada; G.D.O. Lowe--UK

The number of attendees of this subcommittee meeting was approximately 200.

Dr. L. Iacoviello presented the planned activities of the SubCommittee for the next year. Major issues were:

- Preparation of Guidelines for planning studies of genetic epidemiology
- Information on genetic polymorphisms available in "candidate" genes for haemostasis and thrombosis, on their functionality, on the protocols for their determination and on the validation of genotyping
- Standardisation of genetic polymorphism nomenclature
- Registry of on-going studies on the association between genetic and biochemical haemostatic variables and cardiovascular disease
- Development of a WEB page on our Subcommittee, in connection with the ISTH Web-page (<http://www.negrisud.it/ssc> )
- Systematic meta-analysis of the studies on predictive genetic and biochemical haemostatic variables in cardiovascular disease
- Preparation of Guidelines for blood management for homocysteine evaluation.

The first session of the meeting was focused on different methodological approaches to study the association between genetic haemostatic variables and ischaemic vascular disease.

### **Ongoing meta-analysis on biochemical predictive Haemostatic Variables in Cardiovascular Disease.**

Dr. G Lowe underlined the necessity for meta-analysis to include a minimum of 1000 CHD cases in prospective studies, to consider the effect of confounders and the nature of samples to be analysed for antigen levels of haemostatic variables. In the framework of the British Regional Heart Study, they found a strong correlation between D-dimer, von Willebrand factor (vWF), and t-PA antigen levels measured in paired citrated plasma and serum samples. However, carefullness is still necessary in the interpretation of these results, until validation by further studies will be available. In contrast, their self-association over 5 years was lower and needs evaluation in further studies. Results of meta-analyses for fibrin D-dimer, vWF, and t-PA as markers of coronary risk were presented. They all included more than 1,000 CHD cases and showed a significant association with the risk of CHD. Adjustment for confounders did not modify the association for D-Dimer and vWF, while the association with t-PA decreased. A

Fibrinogen Trialist Collaboration has been started to perform meta-analyses including more than 10,000 cases.

**Methodological approaches for association population-based studies in genetics.** Dr. A. Di Castelnuovo presented the use of association population-based studies in genetics. Association studies are based on linkage disequilibrium (LD), a population-based concept identified by noting that certain alleles of two genes occur together in the population more often than by chance.

A major limitation of the approach is confounding by population stratification that can occur in ethnically mixed populations. A way to measure and correct for stratification has been recently proposed, by genotyping a moderate number of genetic markers unlinked with the disease and using the average of association across the unlinked markers as a direct measure of stratification.

Correct sample size estimation is of fundamental importance, especially in testing for gene-environment interactions. Methods to calculate sample size in the presence of interactions have been reviewed. Interaction must be supported by statistical tests, and not solely suggested by data. Synergy index (S) is a useful measure of interaction in case-control studies.

Relevant references to the topic are:

1. MJ Khoury et al. *Fundamentals of Genetic Epidemiology*, Oxford University Press, 1993
2. KJ Rothman et al. *Modern Epidemiology*, Lippincot-Raven, 1998
3. J Ott et al. *Am. J. Hum. Genet.* 67:289-294,2000
4. JK Pritchard et al. *Am. J. Hum. Genet.* 65:220-228,1999
5. ER Reich et al. *Genetic Epidemiology* 20:4-16,2001
6. WD Dupont et al. *Controlled Clin. Trials* 11:116-128,1990
7. SJ Hwang et al. *Am. J. Epidem.* 140:1029-1037,1994
8. I Foppa et al. *Am. J. Epidem.* 146:596-604,1997
9. M Lundberg et al. *Epidemiology* 7:655-656,1996
10. A Di Castelnuovo et al. *Statistica* 2001, in press
11. J Hoh et al. *PNAS*; 97:9615-9617,2000

LE Mitchell et al. *Genet. Epidem.* 19:193-201,2000

**The Utility of Family Studies for Understanding the Genetics of Thrombosis.** Dr. J. Blangero provided an overview of a method for linkage analysis in extended pedigrees known as Variance Component Method (VCM). VCM is a powerful strategy to map genes related with quantitative trait loci (QTL) involved in pathogenesis of complex disease. VCM used data from large pedigrees containing more than 20 individuals in more than 3 generations. The method is based on a decomposition of the variability of the QTL in genetic and environmental causes. He showed that for a disease with large prevalence (35%), the VCM method is more powerful than other linkage-based strategies.

The author presented also some results of the GAIT project, a project aimed at identification of QTL involved in thrombosis, that made use of the VCM strategy in analysing data from Spanish families.

**Twin studies to evaluate the genetics of haemostatic variables.** Dr. P Grant presented examples on how to define the genetic components of single factors related to a syndrome, such as the insulin resistance syndrome. The inheritability of these factors has been suggested by studies on offsprings of probands with diabetes, which showed increased levels of factors related to the metabolic syndrome (including haemostatic factors) as compared with those of the normal population. He presented results on 501 twin pairs that showed a high inheritability of F VII, F XII, F VIII, fibrinogen, vWF, PAI-1, F XIII levels. Moreover, also levels of activation markers of coagulation and fibrinolysis, as such as D-Dimer, prothrombin fragments, thrombin-antithrombin complex, showed a significant inheritability. These results were confirmed by the Leeds Family Study on 537 subjects from 89 families. However, when the most studied polymorphisms in haemostatic genes were tested in these models, they accounted only for a very small percentage of the total inheritability.

**The CANVAS project and the candidate gene approach .** Dr. F Cambien presented the "candidate gene approach" to understand the genetic component of ischaemic cardiovascular disease. The strategy consists in identifying all the possible candidate genes and all the possible relevant polymorphisms in functional parts of the genes. 300-600,000 single nucleotide polymorphisms in regulatory portions of the genes could be expected. It is necessary to genotype all the polymorphisms of a certain gene, since the functional one could be not in linkage disequilibrium with the others (the example of Apo A1 has been given). Dr Cambien then presented the GENE-CANVAS project. The project started in 1998 with the aim of screening for polymorphisms in candidate genes for ischaemic cardiovascular disease, providing assay conditions, allele and genotype frequencies and linkage disequilibrium of identified polymorphisms, and testing associations with ischaemic cardiovascular disease. At 2001, 29 studies, 102 gene and 454 polymorphisms have been analysed and are available at the Web site <http://genecanvas.idf.inserm.fr> .

The second session of the meeting was focused on differences in the association between levels of haemostatic and inflammatory variables and the risk of ischaemic vascular disease.

**Homocysteine evaluation: problems and predictive value.** Dr. M Cattaneo could not be present at the meeting.

**Inflammation variables in the prediction of cardiovascular disease.** Dr. Klufft reported on the 'Leiden Meeting on Inflammation and Cardiovascular Disease'. Information is available at the web site [www.haemost.nl](http://www.haemost.nl) and published in the Italian Heart Journal 2001;2:155-199. Three inflammatory markers have been discussed: CRP, SAA and SFLA2. CRP seems to perform better, since it presents very limited preanalytical problems, has a reliable assay, is widely available and can be considered as both a marker and a functional parameter. It is a systemic marker, but is also localised at the site of atherosclerotic plaques.

The intraindividual variation of CRP levels is about 30%. An upper quartile of PCR level >3mg/L has been established as a cut-off for high CAD risk.

Finally, treatment options have been discussed. The DALI study showed that atorvastatin treatment is able to strongly reduce CRP levels at the dose of 80 mg/day. The dose response for effects on CRP levels was not parallel with those of cholesterol lowering, suggesting that the reduction of CRP was not dependent on the effect of atorvastatin on cholesterol. Evaluation of cost/effect benefit of statins in primary prevention showed an acceptable cost/benefit for people over 45 years.

**Inflammatory markers and sexual hormones.**Dr. Cushman presented an overview of the studies on the effect of hormone replacement therapy (HRT) on CRP levels. HRT doubles the levels of CRP and this effect could explain the results of the two trials that showed an increased risk of CAD after HRT. On the contrary, tamoxifen therapy reduced CRP levels. In the HERS trial, the increased risk of CAD in the first months of follow-up was associated to an increase in the levels of CRP.

**The VITA project: haemostatic variables and prediction of deep vein thrombosis and arteriosclerosis progression.**Dr. Tosetto presented the VITA study (1993 to 1997), a cross-sectional randomised study of 15,109 subjects in the area of Vicenza, Italy. The principal aim of the study was to define the prevalence and the impact of haemostatic variables on the thrombotic risk. For each subject recruited into the study information on family structure, history of thrombosis and DNA samples are available. The main results of the study confirmed the association of APC resistance with the risk of VTE and did not support an association of prothrombin polymorphism with VTE.

Secondary aims were the prospective evaluation of the risk and a cross-sectional evaluation of the impact of haemostatic variables on arteriosclerosis (evaluated as IMT of the common carotid artery) in a subsample of 2,000 subjects. The results showed an association between high fibrinogen levels and the risk of both high IMT and incident myocardial infarction. The VITA study is also part of a European collaborative study on the genetics of thrombosis, the GENERALE project.

The final discussion pointed out that the different methodological approaches to genetic studies are complementary; that genetic studies may be relevant in the understanding of the physiopathology of haemostasis and in defining the genetic components of haemostatic variables levels, rather than the risk of CVD; and that it is important to determine genetic variables together with the circulating levels of the corresponding proteins.

## Predictive Haemostatic Variables In Cardiovascular Disease

July 20, 2002

8:00 to 12:00

Terrace Room

Boston Park Plaza Hotel

Chairman: L. Iacoviello, Italy

Co-Chairs: M. Cushman, USA; P. Grant, UK; R. Hull, Canada; G. Lowe, UK

The number of attendees of this subcommittee meeting was approximately 100.

**Dr. Licia Iacoviello** gave an update of the activities of the Subcommittee during the past year. A WEB site of this SubCommittee has been developed at <http://www.negrisud.it/ssc> and linked with the official ISTH website.

The Registry of on going studies on the association between genetic and biochemical haemostatic variables and cardiovascular disease has been started and the forms to apply are now available at the above website. The forms can be completed and sent to Dr. Iacoviello directly through the website.

The Subcommittee's website is also linked to the CANVAS website ( <http://genecanvas.idf.inserm.fr/> ) which was presented by Dr. Cambien at last year's SSC meeting in Paris. CANVAS has the objective to facilitate the study of the impact of candidate gene polymorphisms on common cardiovascular disorders by accelerating the communication of information on candidate genes and DNA resources. It is also a single nucleotide polymorphism (SNP) resource that may be useful to those exploring the genomic regions where the candidate genes are located. In the next 5 years, 300 candidate genes are foreseen to be included in the catalogue. At the moment information on 566 polymorphisms, 114 genes and 29 studies are available.

Dr. Iacoviello also reported on the ETRO Working Party meeting on Population Genetics of Haemostatic Risk Factors for Arterial Vascular Disease that was held in Rome, on October 26-27, 2001. The aim of this Working Party is to bring together European investigators in genetics, environment and thrombosis with different expertise (molecular and cell biology, epidemiology, biochemistry) to evaluate the contribution of genetic and environmental components to the risk of arterial thrombosis, with a special emphasis on haemostatic factors. The hot topics of this year's meeting were pharmacogenetics and innovative epidemiological approaches, with particular attention to large cohort studies.

Finally it has been proposed to write guidelines for population association studies in genetics. Potential topics are: selection of controls, sample size, multiple comparisons, statistical analysis (permutation test), admixture bias, gene-environment interactions, selection of polymorphisms.

*Are D-Dimer levels a suitable marker of cardiovascular disease? Methodological and epidemiological aspects.* **Dr. Gordon Lowe** presented an overview of fibrin D-dimer in prediction of cardiovascular disease. While there are a large number of available commercial

assays which give very different values for plasma D-dimer, its association with cardiovascular risk appeared consistent regardless of assay type. (Standardization of D-dimer assay is being performed by the Subcommittee on Fibrinolysis). Variation on sample storage, and on repeated measurement after 4 years, appeared minimal. Epidemiological association of D-dimer included age, female sex, country, oral estrogen use, obesity, lack of leisure activity, varicose vein, and prevalent arterial disease or venous thromboembolism. A meta-analysis reported the relative risk of coronary heart disease as 1.7 (top third of D-dimer compared to bottom third). There were fewer studies of the predictive value of D-dimer for stroke or venous thromboembolism, but these showed consistent association with risk. These associations were not explained by acute phase reaction (e.g. C-reactive protein). D-dimer levels are normalised by oral anticoagulant therapy and merit evaluation in selection of patients (and in monitoring) of such therapy.

*Homocysteine Evaluation: Problems and Predictive Value.* **Dr. Marco Cattaneo** reported on the association of homocysteine levels and risk of cardiovascular disease (CVD). While the association between homocysteine and venous thromboembolism is well established, only retrospective case-control studies or prospective studies in patients who already had an ischaemic event demonstrated a consistent association between homocysteine and CVD. In contrast, prospective studies in healthy subjects gave negative results. As a consequence a huge debate is going on whether high homocysteine levels are cause or consequence of atherosclerosis. A study in patients with stroke demonstrated that, following the acute phase, there is a decrease in homocysteine levels, concomitant with the increase in C-RP levels; at longer term, homocysteine levels got back to normal levels, never increased. Although a final conclusion on this debate can only be derived from interventional studies, lowering homocysteine levels (whose results are expected in the next few years), there are still some arguments for a role of homocysteine in the risk of cardiovascular disease. Probably to express its risk potential, homocysteine requires to synergise with other risk factors. Moreover, in prospective studies, the power of the association between homocysteine and CVD decreases with the length of follow-up, suggesting problems related to a longer sample storage. Homocysteine levels can be determined by genetic (MTHFR polymorphism) and environmental factors (vitamins). While there is no consistent evidence of an association between the MTHFR polymorphism and CVD risk, there is evidence for an independent association with vitamin B6.

Dr. Cattaneo also reported on the standardization of pre-analytical conditions for homocysteine evaluation. Homocysteine levels are stable over 6 hours in samples collected in both ACD and EDTA when stored on ice. After storage at room temperature, homocysteine levels increase about 13% if collected in EDTA and only 3%, if collected in ACD. Therefore, when room temperature storage cannot be avoided, ACD must be used as an anticoagulant.

*The ARIC Project: Haemostatic Variables, their genetic control and Prediction of ischaemic arterial disease.* **Dr. Kenneth Wu** presented an overview of the results of the ARIC study, a prospective investigation of a USA cohort of 15,792 healthy subjects aged 45-64 years who were followed for 10 years. A nested case-control study was performed by comparing 365 incident cases and 734 non-cases. Fibrinogen, factor VII, factor VIII, von Willebrand factor, protein C, APC resistance, antithrombin III, t-PA, PAI-1, plasminogen, activated factor VII, and XII, Prothrombin fragment F1+2, D-dimer, beta-thromboglobulin and soluble thrombomodulin levels were evaluated. Fibrinogen, plasminogen and D-dimer levels were significantly and

independently associated with the risk of ischaemic events. Unexpectedly, sTM showed a negative association with the risk of CVD. No associations were found for the other haemostatic factors evaluated. Combined analysis of pro- and antithrombotic factors can provide a more precise estimation of the association: indeed, increased levels of ICAM-1, fibrinogen, F VIII, and vWF interact with low levels of sTM in increasing the risk of CVD, while in the presence of high levels of sTM they did not affect the risk.

Polymorphisms in several haemostasis factor genes (fibrinogen, F VII, F II, F V, F XIII, TM, platelet glycoprotein IIIa and Ib) were also evaluated in a larger population of 800 cases and 900 controls. Only TM and GP Ib polymorphisms were associated with the risk of CVD; however, the association between TM polymorphisms and CVD risk was present in African American subjects but not in Caucasians. This suggests that different ethnic populations should always be analysed separately.

*Ethnic differences in genotype distribution and risk of cardiovascular disease: the case of the Japanese*

**Dr. M. Murata** further discussed ethnic differences in genotype distribution by presenting the case of the Japanese population. In Japan the incidence of venous thrombosis after surgery and myocardial infarction is lower in both males and females, despite the high prevalence of smoking. Some polymorphisms related to such disease (F V Leiden, prothrombin, F XIII, GP Iib/IIIa) are absent in the Japanese population, while many others have a different frequency as compared to the Caucasian population. Some of them are increased, while some others are decreased, without any apparent relation with the potential risk effect. While the reduced incidence of venous thrombosis can at least in part be related to the absence of related polymorphisms, the difference in AMI is related more to different environmental habits, especially diet and to a different pathogenesis. Indeed, 52 % of AMI in Japan can be attributed to coronary spasm, compared to 11% in Europe. Independent predictors of spasm in Japan are smoking and NO synthase polymorphisms.

Dr Murata also showed some results concerning PAF acetylhydrolase gene in a Japanese population. This enzyme inactivates PAF. A F/V polymorphism is associated with plasma activity of the enzyme, showing the VV genotype higher activity, FF no activity and VF intermediate activity. Carriers of VV genotype also showed reduced platelet activation after PAF, but not after ADP or collagen stimulation. The F genotype was associated with an increased risk of stroke, particularly at younger age.

*Activation markers of coagulation and fibrinolysis in twins: heritability of the prothrombotic state.* **Dr. Robert Ariens** reported on the heritability of the prothrombotic state. Heritability is the proportion of variance in a phenotype due to additive genes. Twin studies are a useful tool to evaluate the heritability of a phenotype by comparing monozygotic twins (100% genetics) with dizygotic twins (50% genetics). The levels of F VII, FXII, F XIII, Fibrinogen, vWF and PAI-1 showed a high inheritability. This result was consistent with those obtained in family studies. However, new polymorphisms in genes related to these phenotypes only account for a small part of their heritability (e.g., PAI-1 4G/5G polymorphism accounts for 2% of PAI-1 level heritability, Fibrinogen beta chain polymorphism for 1% of fibrinogen heritability and F VII Arg353Gly polymorphism for 16% of F VII levels heritability). In a sample of 115 twin pairs (59

MZ and 56 DZ), it has also been demonstrated that the levels of coagulation activation markers are also inherited. In particular, prothrombin fragment F1+2, TAT and D-dimer levels showed a degree of heritability of respectively 45, 40 and 65%. Also fibrin structure shows a 39% heritability, although the effect of environmental factors is also important. Among others, glycosilation can be an important determinant of fibrin structure in diabetic patients.

*Genetic regulation of Factor VII and Factor XII: insights from the GAIT Study.* **Dr. John Blangero** provided an update of the results of the GAIT study, a project aimed at identification of QTL in large pedigrees of Spanish families. He suggested that, before studying the genetics of discrete traits (diseases) that require very powerful methods, a useful approach is to identify genetic loci that regulate continuous traits (factor levels), which are called QTL (quantitative trait loci). The study of large pedigrees is one of the more powerful tools for this purpose and allows both the localization and the identification of a QTL.

The GAIT study includes 398 subjects from 12 thrombophilic and 9 randomly selected families, with a total of 57 cases of thrombosis. F VII and F XII liability has been studied. In a first phase, QTL F XII and F VII have been identified by using a wide genome scan with 363 genetic markers. High lod score (LD) has been found in chromosomes 5, 10 and 2 for F XII levels. Chromosome 10 contains F XII gene, however, in chromosome 5 there should be another gene strongly influencing F XII gene. Since a 46C/T polymorphism in F XII gene has been associated with the levels of F XII, if whether such a polymorphism could be responsible for the large linkage signal found has been tested. After conditional analysis, the LD for chromosome 10, although decreased, remained still significant, suggesting that other polymorphisms can be relevant.

For F VII levels a significant QTL has been found in chromosome 13, that actually contains F VII gene. F VII gene was resequenced: all its polymorphisms (49) were identified with an allele frequency between 0.01 and 0.37. Posterior probability of functionality was analysed and 7 polymorphisms were identified as functional. Conditioned analysis including these polymorphisms showed that the LS for F VII levels was reduced to a non-significant level, suggesting that F VII gene was completely dissected.

Finally, Dr. Blangero commented on the limitation of the case-control approach for genetic studies, based on linkage disequilibrium (LD) mapping. Indeed, LD is too unpredictable and, therefore, negative studies give no information.

*Genetic polymorphisms of haemostatic factors: a word of caution.* **Dr. Pier Mannuccio Mannucci** discussed the need to use large samples in case-control studies to have enough power to detect association for polymorphisms in haemostatic genes. He presented the results of a case-control study performed in collaboration with the ANMCO (Italian Association of Hospital Cardiologist), including more than 1000 patients with AMI at young age (under 45 years) and a corresponding number of healthy controls matched for age and sex. Cases differed from controls for all common environmental risk factors, such as smoking, hypertension, diabetes, dyslipidemia, etc. Cases also had a higher prevalence of family history of AMI in respect to controls; however, this difference was not explained by any of the polymorphisms of haemostatic genes studied. Indeed, no associations were found between F VII, FV, F II, F XIII, GP IIIa, etc.,

polymorphisms and the risk of AMI at young age. The same results were obtained when only females were taken into consideration.

**Conclusion:**

The subcommittee meeting highlighted the need to continue to study the role of haemostatic variables in the prediction of cardiovascular risk. Activation markers such as D-dimer levels could be particularly relevant, and their evaluation, also in relation to other well established risk factors, should be introduced in future epidemiological studies. On the other hand, new haemostatic markers are emerging as possibly predictive, such as plasminogen and soluble thrombomodulin and need to be tested in future studies.

A possible powerful approach emerges for genetic studies:

1. QTL identification in family studies
2. identification of all possible polymorphisms of the genes related to the QTL
3. test for their functionality
4. dissect residual inference
5. assess the absolute or relative risk in population studies.
6. design the population study with a correct calculation of the sample size, selection of cases and controls and respecting the homogeneity of populations.

## **Predictive Haemostatic Variables In Cardiovascular Disease**

**July 12, 2003**

**09:00 to 13:00**

**Hall 11**

**The International Convention Center, Birmingham**

Chairman: L. Iacoviello, Italy

Co-Chairs:; P. Grant, UK; G. Lowe, UK; V. Salomaa, Finland; A. Tassetto, Italy

The number of attendees of this subcommittee meeting was approximately 100.

Dr. Licia Iacoviello gave an update of the activities of the Subcommittee. In particular, the WEB site of the SubCommittee at <http://www.negrisud.it/ssc> , linked with the official ISTH website and the registry of on-going studies on the association between genetic and biochemical haemostatic variables and cardiovascular disease. Dr Iacoviello invited the members in the audience who has such studies on-going to fill the forms that are available at the website. The forms can be filled and sent to Dr. Iacoviello directly through the website.

In the first part of the meeting two debates were presented on hot topics related to risk factors for cardiovascular disease: C reactive protein and homocysteine.

### **Should measurements of C reactive protein be done to assess risk in primary prediction/prevention of cardiovascular disease? *C. Kluft – pro; GDO Lowe – against***

Dr. Kluft and Dr. Lowe presented during 10-minutes arguments respectively pro and against the possibility that CRP could be used to assess risk in primary prediction of cardiovascular disease. These presentations were followed by a 10 minutes rebuttal of each discussant and by a general discussion. The debate on C reactive protein and CVD risk will be published by the Journal of Thrombosis and Haemostasis, as an activity of the Subcommittee.

### **Hyperhomocysteinemia should (not) be looked for and treated in patients with cardiovascular disease? *M. Cattaneo – pro; I. Pabinger – against***

Dr. Cattaneo presented evidence from several studies and recent metanalysis on the association between high homocysteine levels and risk of cardiovascular disease; in particular he showed that only in retrospective case-control studies or prospective studies in patients who already had an ischaemic event, a consistent association between homocysteine and CVD was demonstrated. In contrast, prospective studies in healthy subjects gave contrasting results. This discrepancy could be explained by the weak design of the latter studies, by methodological problems in homocysteine evaluation and to the too long follow-up. Although there is a clear association between homocysteine and secondary cardiovascular disease, a criticism is often raised that a

casual relationship between homocysteine and CVD has not been proven. In particular, there is no clear evidence that by reducing homocysteine levels one may decrease the risk of cardiovascular disease.

Dr. I. Pabinger pointed the attention on the lack of studies proving the therapeutic effectiveness of lowering homocysteine. In particular, out of the only two studies published until now on the use of folic acid to decrease CVD, one was positive, and one was negative. Moreover, the possibility that folic acid treatment can mask vitamin B12 deficiency should be taken into consideration. She proposed that the best attitude is to await the results of coming studies on the effect of lowering homocysteine by eating more fruit and vegetables.

### **Is "candidate" gene still a useful approach to study the genetics of cardiovascular disease?**

*A. Catto*

Dr. A. Catto gave an overview of population association studies on the genetics of CVD using the approach of candidate genes in relation to the field of haemostasis. For all candidate genes studied contrasting results have been published and it is difficult at the moment to establish an unequivocal association with the risk for any of them. He examined the possible causes of such a disappointing finding. The small sample size of the studies, the bad definition of clinical phenotypes, the incorrect selection of control populations, the lack of linkage disequilibrium between markers and functional polymorphisms may have the main role. However a recent publication by performing a metaanalysis of several candidate genes associated to a number of different clinical phenotypes demonstrated that the candidate gene approach is indeed able to detect genetic associations, although the extent of the association is small.

### **Preparing a reference genetic (gDNA) panel for Factor V Leiden. E. Gray**

Dr. Gray presented the first DNA standard obtained by human gDNA to be used in factor V Leiden evaluation. All people interested to participate in collaborative studies can contact Dr. Gray ([egray@uibsc.ac.uk](mailto:egray@uibsc.ac.uk)).

### **Genetic markers of cardiovascular risk answers from large cohort studies. V. Salomaa**

Dr. Salomaa presented the disadvantages reported in studying genetic association in case-control studies: small studies, publication bias, cross-sectional design, only one or two polymorphisms studies in single genes. Large multinational cohort studies coupled with high throughput genotyping can overcome this problem. He presented as an example the MORGAM study (<http://www.ktl.fi/morgam>). This is a case-cohort design on 2000 incident CHD events and 4000 matched controls, using a candidate gene approach. Multiple gene and multiple polymorphism in single gene will be genotyped: 500 SNPs in 60 genes related to coagulation and inflammation, 150 SNPs related to integrins and 130 SNPs in 63 genes related to platelet receptors and

infection. SNPs have been selected according to the following principles: candidate genes, systematic approach of certain biological pathways, all SNPs found within or at close proximity, rare allele frequency higher than 1%, linkage disequilibrium, SNPs in evolutionary conservative area according to comparative genomics, density of one SNP per 5 Kb. Finally he presented preliminary results relative to thrombomodulin (TM) gene pathway in the FINRISK Study (that will be part of the larger MORGAM Study). The FINRISK is a population survey study started in 1992 on 5299 subjects, 25-64 years old, followed-up until 2000. 172 incident CHD, 109 stroke and 257 death were observed. Preliminary results show association between different polymorphisms in TM gene and either risk or protection of CVD.

**Do markers to predict drug efficacy in thrombotic disease exist? The case of aspirin. J. Musial**

Dr. Musial presented data on the possible genetic modulation of aspirin activity in an in vivo model of thrombin formation. 300 mg of aspirin prolong bleeding time and decrease thrombin generation measured as fibrinopeptide A (FPA) concentration in bleeding time blood. When this experiment was performed in subjects homozygous for P1A1 allele or carriers of the P1A2 allele of the GpIIIa platelet polymorphism different results were observed. When the effect of aspirin was compared to that of clopidogrel, opposite results were observed. Indeed, while the effect of aspirin on bleeding time and thrombin generation was reduced in P1A2 carriers, the effect of clopidogrel on the same system was reduced in P1A1 homozygous. Finally Dr. Musial showed how the effect of aspirin in inhibiting activation of FXIII was modulated by a polymorphism (Val34Leu) in FXIIIa subunit gene.

**A brief update on the fibrinogen study collaboration. GDO Lowe**

Dr. Lowe gave a brief update on Fibrinogen Studies Collaboration. This is a meta-analysis on the relationship between fibrinogen and CVD in prospective studies, with the main aim to assess: associations, potential confounders, regression dilution, shape of dose-response relationship.

Until now 160.000 persons in 28 out of 30 studies have been considered. Interested people can contact the coordinator of the study, Prof. John Danesh at [jd292@medschl.com.ac.uk](mailto:jd292@medschl.com.ac.uk)

## Predictive Haemostatic Variables In Cardiovascular Disease

June 19, 2004

8:30 to 12:30

Cipressi Room

Fondazione Giorgio Cini

Chairman: P.J. Grant, UK

Co-Chairs: L. Iacoviello, Italy; G. Lowe, UK; V. Salomaa, Finland; A. Tassetto, Italy

The session was co-chaired by Drs Iacoviello and Lowe, Dr Grant had sent his apologies.

**Dr. Lowe** gave an overview of haemostatic variables in prediction of cardiovascular (arterial) events – principally coronary heart disease (CHD) events in meta-analyses of prospective studies. Currently, fibrinogen, von Willebrand factor antigen, fibrin D-dimer, tissue plasminogen activator antigen, and possibly factor V Leiden and prothrombin 20120 mutations, showed significant associations in such analyses. Further data are required for other haemostatic variables including factors VII, VIII and IX; PAI-1, and other activation markers. The Fibrinogen Studies Collaboration (FSC) is analyzing individual data from 200,000 persons in prospective studies of plasma fibrinogen; this should clarify the shape of the dose-response curve, the effect of assay type, and the additional predictive value of fibrinogen to that of classical CHD risk predictors. The Emerging Risk Factors Collaboration (developing from the FSC) aims to perform similar collaborative meta-analyses on other circulating potential risk predictors, starting with C-reactive protein, lipoprotein (a) and albumin. Action: Dr Lowe to draft brief report of overview; and to liaise between Subcommittee and Emerging Risk Factors Collaboration on future analyses of haemostatic variables.

**Dr. G. Palareti** (Italy) reviewed the predictive value of haemostatic variables for recurrence of venous thrombosis. Currently, D-dimer, factor VIII and factor IX appeared of potential clinical value; further studies were required. Dr Palareti outlined the ongoing multicentre, Italian PROLONG study of D-dimer, assayed one month after discontinuing oral anticoagulation, in prediction of the need for prolonged therapy.

**Dr. D. Fitzgerald** (Ireland) reviewed the potential for platelet proteomic studies to identify markers of cardiovascular risk. Many platelet proteins had roles in inflammation, rather than haemostasis. There remained many methodological challenges, including quantification of proteins. Recently-developed antibody arrays allowed some quantification of platelet cytokines and growth factors.

**Dr. A. Carter** (UK) reviewed the potential for plasma proteomic profiles to identify cardiovascular risk. There are about 500 proteins which function in plasma, and another 50,000 which are markers of cell secretion, death or damage. There is a wide dynamic range (10 to power 10) of currently measured plasma proteins (from albumin to interleukin-6), and 10 to power 7 for haemostatic proteins. The Human Proteome Organisation (HUPO) Plasma Protein Project is assessing issues including variants, population distributions, and the effects of age, sex, lifestyle, drugs and diseases. Current activities include assessment of technology platforms, pre-

analytical variables, and methods for depletion of the 6 most abundant plasma proteins. Different methods are required for classic plasma proteins, tissue damage markers, tumour markers and cytokines. Complex bioinformatics is required for analyses of 2D electrophoresis data. There is a need for standards to run in epidemiological studies. Dr Carter presented some illustrative data on clot proteomic analyses. Action: Drs Carter to liaise with Dr Fitzgerald to draft brief report of overviews; and to liaise between Subcommittee and HUPO-Plasma Protein Project for haemostatic variables.

**Dr. Iacoviello** presented new perspectives in studying the associations between genetics and cardiovascular disease. We have studied until now only individual polymorphisms in single genes. However, the picture is much more complex.

Several polymorphisms have been discovered in each single gene and, since the linkage disequilibrium principle has been invalidated, we should in principle measure all the identified polymorphisms in our studies. However, an analytical method has been recently developed to identify combinations of polymorphisms that can be studied in haplotypes to cover about 80% of the genetic information contained in a gene.

Linkage analysis in large families, by using a wide genome scanning approach, can also be useful for further approaches. The latter implies the identification of genomic areas associated with QTL and, through QTL, with thrombosis. Action: De. Iacoviello to draft brief report of overview.

Numbers attending session were estimated as 60-80.

## **Predictive Haemostatic Variables In Cardiovascular Disease**

**6 August 2005**

**11:00 to 14:30**

**Pyrmont Room**

**Sydney Convention and Exhibition Centre**

Chairman: P.J. Grant, UK

Co-Chairs: G. Lowe, UK; G. Palareti, Italy; V. Salomaa, Finland; A. Toso, Italy

**Audience: between 80-100 participants**

**Update on meta-analyses of fibrinogen, CRP, IL-6, vWF,tPA and D-dimer in prediction of vascular risk. Professor G.D.O Lowe, Glasgow , UK .**

This talk reviewed a metanalysis of 31 studies of fibrinogen and vascular risk involving ~ 154,000 individuals. The methods involved have been previously published (Eur J Card Prev Rehab, 2004). A 3 fold increase in CV risk was seen in the top 20% of fibrinogen levels. Similar relationships existed between fibrinogen levels and both stroke and non=vascular deaths. Neither the fibrinogen assay used by the investigators, nor time to assay affected the results.

CRP had a weaker association with vascular risk than fibrinogen and seemed to show more variation with storage than fibrinogen. Other variables had weaker associations with CV risk

**Proteomics and Haemostasis: An update on the plasma clot proteome. Dr A. M. Carter . Leeds UK .**

The relative information gained by examining the genome, transcriptome and proteome was discussed. The plasma proteome is difficult to characterise, there is a wide dynamic range of proteins with albumin alone accounting for 55% of proteins in plasma. The Human Proteome Organisation was discussed and the aims and objectives of the plasma proteome project outlined. Standardisation of methodologies is a problem and reference samples are being developed and sent out to participating laboratories.

**Increased numbers of circulating endothelial cells predict poor outcome in acute coronary syndromes. A. Blann, Birmingham , UK**

The potential for measurement of circulating endothelial cells (CECs) as a marker of vascular disease was discussed. CECs are recognised by the presence of CD146 and generally exist in concentrations of ~ 1 cell/ml of venous blood. In acute coronary syndromes this may rise to ~20 cells/ml of venous blood. Increased CECs have also been described in a variety of inflammatory states.

Methodological issues were discussed, in particular the problem of differentiating CECs from circulating progenitor cells, a problem that doesn't appear to have been entirely resolved.

**The adipocyte and cardiovascular risk. J. Prins, Brisbane , Australia**

Recent work on the paracrine and endocrine effects of the fat filled visceral adipocyte was presented. The cardiovascular system was described in novel terms of obeying the messages sent out by the adipocyte and the brain. The obese adipocyte secretes 3 times as much TNF alpha and also secretes increased renin angiotensin activity, PAI-1, leptin, with suppressed adiponectin. The structure function of adiponectin and its receptors was discussed.

**Multiple Environmental and Genetic assessment of risk factors for venous thrombosis, the MEGA Study. C. Doggen. Netherlands**

The MEGA study design was presented with preliminary data from this cohort. Interactions between FV Lei.den and contraceptive use was presented and the analysis of the data set was discussed.

*P.J. Grant Sydney, August 2005*

## Predictive Variables in Cardiovascular Disease

Chairman: P.J. Grant, UK

Co-Chairs: G. Lowe, UK; V. Salomaa, Finland; A. Toso, Italy

Around 50 people attended the session

1. *Professor Peter Grant* ( Leeds, UK), as outgoing chairman, introduced the meeting by summarising some of the activities of the sub-committee and by providing a brief overview of the complexities of the cardiovascular disease phenotype.
2. *Professor Niko Marx* ( Ulm, Germany) presented data on the role of various circulating and cellular components of the inflammatory cascade in the pathogenesis of cardiovascular disease. A general discussion followed on the importance of inflammation in these processes and the evidence as to whether inflammation was causative or an ‘innocent bystander’.
3. *Professor Gordon Lowe* ( Glasgow, UK) presented a review of meta-analyses of haemostatic variables and risk of coronary heart disease. The Fibrinogen Studies Collaboration has established that fibrinogen shows an independent association. Weaker associations had been established for von Willebrand factor, fibrin D-dimer, t-PA antigen, Factor V Leiden and the prothrombin mutation. To date, there were no significant associations reported for other haemostatic SNP’s, hence further studies of haplotypes might be preferred.
4. *Dr Angela Carter* ( Leeds, UK) presented a talk on “The Potential of Proteomic Technologies to Discover Novel Risk Factors for Acute Coronary Syndromes”. This talk followed on from previous presentations at the SSC discussing the application of proteomics for identification of predictive variables for cardiovascular disease. The presentation focussed on recent work from Leeds to establish methods for analysing the protein components of ex-vivo formed plasma clots and FXIII substrates. Data were presented from these studies to show that the methodologies employed had so far identified both known and novel clot components. Novel clot components and FXIII substrates included complement proteins, lipoproteins and proteins involved in iron homeostasis. Discussion followed about the details of methodology and the relevance of the novel proteins identified in the context of cardiovascular disease, particularly in light of earlier presentations on the validity of more established inflammatory and haemostatic cardiovascular risk factors. Broader discussions about the future application of proteomics to cardiovascular research also took place.
5. *Dr Moniek de Maat*, ( Rotterdam, The Netherlands) The genetics of Cardiovascular Disease: SNP and Haplotype Analysis
6. *Dr Licia Iacoviello*, Campobasso, Italy The genetics of Cardiovascular Disease: Where Next ?

Both these talks focussed on the potential for genetics in understanding the pathogenesis of coronary artery disease. Both presentations agreed that the use of haplotype analysis provided greater opportunities for rigorous data than SNP analysis alone and some of the theoretical background to the use of haplotype analysis was presented. A lively discussion ensued around

the topic of the clinical utility of genetic analysis and the areas of research in which it would provide valuable information.

### Summary

Professor Grant summarised the meeting and concluded by stepping down as the current SSC chairman

## Predictive Variables in Cardiovascular Disease

Chair: G. Lowe ( UK)

Co-Chairs: P. Grant ( UK), V. Salomaa ( Finland), A. Tosetto ( Italy)

**Drs Lowe and Tosetto** presented a summary of the draft report, prepared by the Chair/Co-Chairs, on Haemostatic Variables in Prediction of Arterial Cardiovascular Events in healthy persons. This was discussed. A further draft will be prepared in October, incorporating the discussion points as well as comments from invited experts. A final draft will be submitted to SSC by end 2007.

**Dr Salomaa** presented a review of thrombomodulin (antigen, activity and genotypes) and their associations with cardiovascular events. Further work was recommended especially on activity and genotypes.

**Dr R Tait** presented a study of fibrin D-dimer and other haemostatic variables in prediction of embolic events in persons with atrial fibrillation. D-dimer appeared to add predictive value to the CHADS clinical score. Further management studies were suggested.

A further report on haemostatic variables in prediction of arterial cardiovascular events in persons with established cardiovascular disease was discussed as a possible future Subcommittee activity.

**Dr G Palareti** reviewed published studies on D-dimer as a predictor of recurrent venous thromboembolic events after discontinuation of oral anticoagulants; and gave an update from the ongoing PROLONG-2 trial. **Dr M Verhovsek** presented a meta-analysis of the published studies of D-dimer as a predictor of such events. **Dr P Meijer** reviewed progress with harmonisation of D-dimer assays in management of VTE, including an update from the Fibrinolysis Subcommittee. After discussion, it was recommended that the Predictive Subcommittee should draft a brief report on the predictive value for recurrent VTE of D-dimer (perhaps including an updated meta-analysis, possibly including individual participant data) and other haemostatic variables, in liaison with the Subcommittee on Fibrinolysis. A further report on haemostatic variables in prediction of first VTE events was discussed as a possible future Subcommittee activity.

# Predictive Variables and Cardiovascular Disease

4 July 4  
Vienna, Austria

Chair: *Gordon Lowe, UK*

Co-Chairs: *James Douketis, Canada; Veikko Salomaa, Finland; Alberto Tosetto, Italy*

## DRAFT

G. Lowe (UK) presented the final Subcommittee report recommendations on associations of haemostatic variables with risk of arterial thrombotic events. This report would now be submitted to the SSC.

J. Douketis (Canada) presented an overview of recurrent venous thromboembolism, emphasizing the need to identify clinical and laboratory predictors of recurrence, which might be used to facilitate clinical decisions on long-term anticoagulant prophylaxis. F. Rosendaal (NL) reviewed clinical predictors, which included male sex, idiopathic presentation, and cancer. These appeared stronger predictors than thrombophilias, although further work on genetic predictors was required. P. Clark (UK) discussed the associations of ABO blood group non-O with thrombosis, including potential mechanisms such as higher levels of the FVIII:VWF complex, and the need for further studies including recurrent thrombosis. O. Wu (UK) presented a systematic review of thrombophilias and recurrent thrombosis. FV Leiden showed a weak association; while further data was required to assess the association of other thrombophilias. J. Douketis (Canada) presented a collaborative meta-analysis of fibrin D-dimer in 7 studies: persistently raised levels after discontinuation of anticoagulants was associated with a 2-3 fold increased risk of recurrence. A. Tosetto (Italy) presented the study design of an ongoing collaborative individual patient meta-analysis of these studies. Results of this subcommittee activity would be presented in Boston in 2009. Possible future subcommittee activities discussed might include collaborations on genetic studies; risk of recurrence in less common thrombophilias; and thrombotic risk factors for recurrent arterial thrombosis.

P. Reitsma (NL) reviewed the genetic architecture of venous thrombosis, as revealed by different types of ongoing studies. A. Carter (UK) reviewed genetic determinants of fibrin structure and function in relation to stroke, including results from the EUROCLOT study. L. Iacoviello (Italy) reviewed the development of studies of genetic associations of coronary heart disease. It was agreed that much further work was required to identify new genetic predictors of thrombotic events, and that it would be useful to have a presentation on new statistical approaches to prediction in Boston in 2009.

*Submitted by G. Lowe*