Heritability of Measures of Clot Turbidity and Lysis in the Leeds Family Study

AM Carter[1], CM Cymbalista[1], TD Spector[2], PJ Grant[1]

On behalf of the EuroCLOT Consortium

[1]Academic Unit of Molecular Vascular Medicine, The LIGHT Labs, University of Leeds, UK.
[2]Twin Research and Epidemiology Department, King's College London, UK

Introduction:

Clot formation and fibrinolysis are dynamic processes and their relative balance is considered to reflect thrombotic potential following vascular injury. Identification of factors influencing clot formation, morphology and lysis may further our understanding of the pathogenesis of thrombosis.

Methods (1):

Turbidimetric Assays:
- 25 µl of citrated plasma (in 96 well plates)
- 75 µl assay buffer (0.05M Tris, 0.1M NaCl, pH 7.4)
- 50 µl of activation mix (final conc 0.03 U/ml thrombin [Calbiochem], and 7.5 mmol/l calcium in assay buffer)
- +12.5 ng of tPA (Technoclone) for lysis assay
- Read at 340 nM every 12s for 1 hour and every 2 mins for subsequent 9 hours (lysis assay only)
- Data analysed for measures of clot structure and function using custom software (figure 1)

Conclusions:

This study indicates that genetic factors contribute to variance in clot structure and function. Since clot formation, morphology and lysis are related to the development of vascular disorders, identification of genetic factors influencing clot structure and function may further our understanding of the underlying factors influencing risk for thrombotic cardiovascular diseases.

Aims:

To: (1) establish high throughput turbidimetric assays for clot structure/function
(2) determine the heritabilities of turbidimetric measures of clot structure/function
(3) determine the contribution of haemostatic factors and candidate polymorphisms to variance of these measures.

Methods (2):

The Leeds Family Study:
- 537 individuals from 89 families (mean 6 members per family)
- Fasting blood samples previously analysed for haemostatic and metabolic factors
- Genotyped for polymorphisms in fibrinogen, FXIII and PAI-1

Statistical Analyses:
- Log transformed data were analysed
- Quantitative genetic analyses performed using SOLAR v2.1.4, adjusting for age and sex

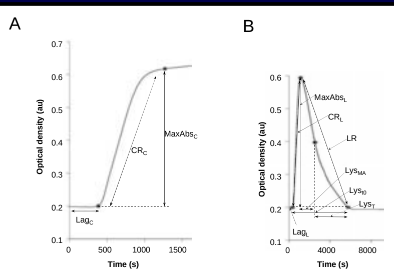

Figure 1. Illustration of the turbidimetric clotting and lysis variables
Panel A. Turbidimetric clotting assay: lag time (LagC) taken as time to exponential increase in absorbance; maximum absorbance (MaxAbsC) taken as absorbance where 3 consecutive readings were identical corrected for the LagC; clot rate (CRC) derived from time and absorbance for LagC and MaxAbsC.
Panel B. Turbidimetric lysis assay variables: lag time (LagL); maximum absorbance (MaxAbsL) taken as highest absorbance value adjusted for LagL; clot rate (CRL); Lys50t0, taken as time at which a 50% fall in absorbance from MaxAbsL occurred; Lys50MA, taken as the time from MaxAbsL to the time at which a 50% reduction in absorbance occurred; LysT, taken as the time from MaxAbsL to the time for the absorbance values to return to baseline; lysis rate (LR) was derived from time and absorbance values for MaxAbsL and the point at which absorbance values returned to baseline; area under the curve (AUC).

Table 1. Mean values and heritability of turbidimetric measures of clot Structure and function

Turbidimetric Variables	Mean (95% CI)	Heritability (95% CI)
Lag$_C$ (s)	316 (310, 323)	0.33 (0.15, 0.51)*
Lag$_L$ (s)	319 (313, 325)	0.31 (0.14, 0.49)†
MaxAbs$_C$ (au)	0.329 (0.321, 0.338)	0.28 (0.13, 0.43)*
MaxAbs$_L$ (au)	0.277 (0.269, 0.285)	0.30 (0.14, 0.46)†
CR$_C$ (x10-4 au/s)	2.51 (2.44, 2.58)	0.17 (0.01, 0.34)*
CR$_L$ (x10-4 au/s)	2.81 (2.73, 2.90)	0.22 (0.06, 0.38)†
Lys50$_{t0}$ (s)	2617 (2566, 2670)	0.32 (0.16, 0.49)†
Lys50$_{MA}$ (s)	1248 (1208, 1288)	0.37 (0.20, 0.55)†
Lys$_T$ (s)	5572 (5341, 5831)	0.26 (0.12, 0.39)†
LR (x10-4 au/s)	0.454 (0.490, 0.472)	0.25 (0.11, 0.39)†
AUC	604 (574, 636)	0.33 (0.16, 0.50)†

* adjusted for age and sex; † adjusted for age, sex and batch
au = absorbance units; s = seconds

Table 2. Proportion of variance in turbidimetric measures of clot structure and function attributable to haemostatic factors and demographic and biochemical variables

Model	LagC*	MaxAbsC*	CRC*	Lys50t0†	Lys50MA†	LysT†	LR†	AUC†
Age, sex, *batch	0.057	0.069	0.047	0.102	0.100	0.120	0.125	0.109
+ fib	0.135	0.478	0.222	0.188	0.184	0.135	0.226	0.338
+ FXIII	-	-	-	0.205	0.197	-	-	-
+ PAI-1	-	-	0.238	0.300	0.307	0.266	0.296	0.440
+ tPA	-	0.484	-	-	-	-	-	-
+ V34L	-	-	-	0.311	0.320	0.279	0.303	0.449
+ BMI	-	0.490	-	0.344	0.341	0.295	0.313	0.468
+ glucose, insulin	0.156	-	-	0.371	0.362	0.302	0.319	0.474

*Adjusted for age and sex; † adjusted for age, sex and batch

EuroCLOT

Presented on behalf of the Euroclot Consortium:

Prof Tim Spector, King's College London, UK (Co-ordinator)
Prof Peter J Grant, University of Leeds, UK
Prof Frits Rosendaal, Leiden University Medical Center, Netherlands
Prof Aarno Palotie, University of Helsinki, Finland
Prof Alun Evans, Queens University Belfast, UK
Dr Antonia Stazi, National Center of Epidemiology Rome, Italy
Prof Nancy Pedersen, Karolinska Institute, Sweden
Prof Jose Manuel Soria, Hospital Santa Creu i Sant Pau Barcelona, Spain

6th Framework Programme
Contract No.LSHM-CT-2004- 005268

LIGHT
Leeds Institute of Genetics, Health and Therapeutics

UNIVERSITY OF LEEDS