Genetics of the anticoagulant drug warfarin

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PREFACE

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text

The dissertation does not exceed the page limit of 300 specified by the Biology Degree Committee

ABSTRACT

The path towards personalised medicine requires understanding how the genetic makeup of each individual patient impacts on drug safety and efficacy. In this thesis I use the most widely prescribed anticoagulant drug, warfarin, as a model to investigate the effect of genetic determinants on drug efficacy and safety. Problematic clinical features of using warfarin include a narrow therapeutic range of PT INR 2-3, inter-individual dose variation of 20 folds and severe bleeding complication in 2% of patients.

Following a literature review of all the genes involved in warfarin pharmacokinetics and pharmacodynamics, 35 candidate genes were selected for investigation. Two independent Swedish cohorts of warfarin-treated patients were analysed. First linkage disequilibrium maps were constructed for each gene. Selected SNPs integrated with putative functional variants were genotyped in 201 patients recruited at the Uppsala University. A panel of 216 haplotype tag SNPs was then derived to analyse an independent cohort of 1496 patients from the prospective Warfarin Genetic study in Sweden (WARG).

The two studies were analysed separately for genetic association to warfarin dose requirement (single marker and haplotypic tests). Common SNPs in the vitamin K epoxide reductase gene (*VKORC1*) are significantly associated with dose in the Uppsala and WARG studies ($p = 1.9x10^{-15}$ and $6.5x10^{-100}$, respectively). Cytochrome P450 2C9 (*CYP2C9*) has been known to affect dose requirement and was confirmed in both Swedish cohorts ($p = 2.3x10^{-5}$ and $4.9x10^{-32}$). The two genes together explain ~40% of warfarin dose variation. SNPs in microsomal epoxide hydrolase (*EPHX1*) and orosomucoid 1 (*ORM1*) genes do not show a broad effect but are associated with dose in both studies. Genes encoding PROC, APOE, CALU, PDIA2 and GGCX showed nominal association with dose in the Uppsala study. Likewise, *PROS1, CYP1A1, CYP3A4, PDIA5, PDIA3* and *F10* showed nominal association to dose in the WARG study. Most of these minor effects, if real, are most likely to be population/treatment specific. A model taking in to account genetic factors (*VKORC1* and *CYP2C9**2 / *3) and non genetic factors (age, gender and drug interaction) together explained more than 50% inter-individual dose variance.

We analysed 64 patients from the Uppsala and WARG studies with recorded severe bleeding episodes using the same 216 common SNPs. Case-control analysis found SNPs in *PDIA4*, *P4HB* and *NR1I3* to be associated ($p \leq 0.01$) with bleeding. Using a recessive model, patients with a gastrointestinal bleeding sub-phenotype in the WARG cohort showed association with common variants in *PDIA6* ($P = 0.0014$, odds ratio = 6.98). We sequenced the exons of 11 of the candidate genes in 36 bleeders and 12 non-bleeders (Uppsala study). However, no high penetrance mutation was discovered.

To my dear parents

Mr Chi-Hwa Chen

Mrs Huei-Wan Liu-Chen

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LIST OF ABBREVIATIONS

A	Adenine
aa	amino acid
ABCB1	P-glycoprotein gene or MDR1 gene
aCGH	Array comparative genomic hybridisation, or array CGH
ADR	Adverse Drug Reaction
ALAT	Alanine aminotransferase
APOC2	Apolipoprotein C-II
APOE	Apolipoprotein E gene
BLAST	Basic Local Alignment Search Tool
bp	base pair(s)
BW	Bodyweight
C	Cytosine
CALU	Calumenin gene
cDNA	complementary DNA
CEPH	Centre d'Etude du Polymorphisme Humain
CEU	Caucasian of European origin
CI	Confident interval
CNV	Copy number variation
CYP	Cytochrome P450
CYP1A1	Cytochrome P450 1A1 gene
CYP1A2	Cytochrome P450 1A2 gene
CYP2C	Cytochrome P450 2C family
CYP2C18	Cytochrome P450 2C18 gene
CYP2C19	Cytochrome P450 2C19 gene
CYP2C8	Cytochrome P450 2C8 gene
CYP2C9	Cytochrome P450 2C9 gene
CYP3A4	Cytochrome P450 3A4 gene
CYP3A5	Cytochrome P450 3A5 gene
dbGaP	Database of Genotype and Phenotype
dbSNP	database of SNPs
DDW	Double distilled water
DMEM	Dulbeco's modified Eagle's medium
DNA	DeoxyriboNucleic Acid
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTI	Direct thrombin inhibitor
DVT	Deep vein thrombosis

EBI	European Bioinformatics Institute
ECR	Evolutional conserver region
EDTA	EthyleneDiamineTetraAcetic acid
ENCODE	the ENCyclopedia Of DNA Elements
EPHX1	Epoxide hydrolase 1, microsomal gene
ER	Endoplasmic reticulum
EST	Expressed Sequence Tag
EU	European Union
F10	Coagulation factor X gene
F2	Coagulation factor II gene or prothrombin gene
F5	Coagulation factor V gene
F7	Coagulation factor VII gene
F9	Coagulation factor IX gene
FBS	Fetal bovine serum
FII	Coagulation factor II or prothrombin
FIIa	Coagulation factor II activated or thrombin
FIXa	Coagulation factor IX activated
FV	Coagulation factor V
FVII	Coagulation factor VII
FVIIa	Coagulation factor VII activated
FX	Coagulation factor X
FXa	Coagulation factor X activated
G	Guanine
G6PD	Glucose-6-phosphate dehydrogenase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GAS6	Growth-arrest specific 6
GGCX	Gamma-glutamyl carboxylase gene
GWAS	Genome-wide association study
Hapmap	International Hapmap Project
HDL	High Density Lipoproteins
HGP	Human Genome Project
HMWK	High molecular weight kininogen
HUGO	Human Genome Organization
HWE	Hardy-Weinberg Equilibrium
Kb	Kilo base pairs
LD	Linkage disequilibrium
MAF	Minor Allele Frequency
Mb	Mega base pairs
MDR1	Multidrug resistance protein 1
MHC	Major histocompatibility complex

mRNA	messenger RNA
NAD(P)H	Nicotine adenine dinucleotide phosphate dehydrogenase
NAT	Arylamine N-acetyltransferase
NCBI	National Center for Biotechnology Information
ncSNP	non-coding SNP
NIH	National Institute of Health
NIH	National Institutes of Health
NQO1	NAD(P)H dehydrogenase, quinone 1 gene
NR1I2	Pregnane X receptor gene
NR1I3	Constitutive androstane receptor
NSAID	Non-steroidal anti-inflammatory drugs
nsSNP	Non-synonymous SNP
OMIM	Online Mendelian Inheritance In Man
OR	Odds ratio
ORM1	Orosomucoid 1 gene or Alpha-1-acid glycoprotein 1 gene
ORM2	Orosomucoid 2 gene or Alpha-1-acid glycoprotein 2 gene
P4HB	Prolyl 4- hydroxylase subunit beta
PCR	Polymerase Chain Reaction
PCR	Polymerase Chain Reaction
PDI	Protein disulfide isomerase
PDIA2	Protein disulfide isomerase family A, member 2
PDIA3	Protein disulfide isomerase family A, member 3
PDIA4	Protein disulfide isomerase family A, member 4
PDIA5	Protein disulfide isomerase family A, member 5
PDIA6	Protein disulfide isomerase family A, member 6
PGx	Pharmacogenetics and pharmacogenomics
PIVKA-II	Proteins induced by vitamin K antagonism
PROC	Protein C gene
PROS1	Protein S gene
PROZ	Protein Z gene
PT INR	Prothrombin time international normalised ratio
PXR	Pregnane X receptor
QC	Quality check or quality control
RFLP	Restriction Fragment Length Polymorphism
RNA	RiboNucleic Acid
RNA	Ribonucleic acid
SAEC	Severe Adverse Event Consortium
SAP	Shrimp alkaline phosphatase
SERPINC1	Anti-thrombin III gene
SJS	Stevens-Johnson Syndrome

SNP	Single nucleotide polymorphism
SNP	Single Nucleotide Polymorphism
STR	Short tandem repeats
T	Thymine
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TSC	The SNP Consortium
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1
US FDA	United States Food and Drug Administration
UTG	UDP-glucuronosyltransferase
UTR	Untranslated region
VKD	Vitamin K dependent
VKOR	Vitamin K epoxide reductase
VKORC1	Vitamin K epoxide reductase complex subunit 1 gene
VNTR	Variable Number Tandem Repeat
vWF	von Willebrand factor
WARG	Swedish Warfarin Genetics study
WHO	World Health Organisation
WTCCC	Wellcome Trust Case Control Consortium

PUBLICATIONS ARISING FROM THIS WORK

Wadelius M, **Chen LY**, Eriksson N, Bumpstead S, Ghori J, Wadelius C, Bentley D, McGinnis R, Deloukas P. (2007). Association of warfarin dose with genes involved in its action and metabolism. *Hum Genet.* 121(1):23-34.

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