

https://dspace.org/

Evaluation of CYP2C9 and VKORC1 gene variants that may result in warfarin dosage sensitivity and poor pregnancy outcomes

Mitchell, Cathrine

2008-10-15T10:23:47Z
http://hdl.handle.net/10539/5757

Downloaded from a DSpace repository. https://dspace.org/

Evaluation of *CYP2C9* and *VKORC1* gene variants that may result in warfarin dosage sensitivity and poor pregnancy outcomes

Cathrine Mitchell

Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine in Human Genetics.

2008

DECLARATION

I, Cathrine Mitchell declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in Human Genetics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



19/03/08

Cathrine Mitchell

Date

DEDICATION

This is dedicated to my family: father Patrick Mitchell; mother Jennifer Mitchell, brothers Gary and Peadar Mitchell, Brad Kurth, Zachary Nkhata and friends. Thank you for all of your support and patience with me throughout my studies.

PRESENTATIONS

- Mitchell C and Krause A. Evaluation of *CYP2C9* and *VKORC1* gene variants that may result in warfarin dosage sensitivity and poor pregnancy outcomes. 12th South African Society of Human Genetics (SASHG) Congress; 2007 Mar 1-3; Golden Gate, Freestate, South Africa. Presented by Cathrine Mitchell

ABSTRACT

Warfarin is the most widely prescribed oral anticoagulant used for the long-term treatment and prevention of thromboembolic events. Its administration is challenging as it may result in bleeding-related deaths, inadequate anticoagulation and fetal teratogenesis, including fetal warfarin syndrome. A number of environmental and genetic factors contribute to interindividual warfarin dosage variability. The *CYP2C9* and *VKORC1* genes explain 40-50% of this variability. The aim of this study was to determine the frequency of known and any new variants in these genes in the SA black population, and correlate these variants and a small subset of environmental factors to dosage variability and pregnancy outcomes. I sequenced the exons and intron/exon boundaries of the *CYP2C9* and *VKORC1* genes in 100 random black control and 113 patient samples that had at least one pregnancy on warfarin. I observed six previously described *CYP2C9* variants, 27 novel *CYP2C9* variants, and three previously described *VKORC1* variants. 14 of these variants were observed at an allele frequency of ≥ 0.02. Of these 14, six appear to decrease (all of which are *CYP2C9* variants) and four increase (2 *CYP2C9* variants and two *VKORC1* variants) warfarin dosage requirement. These 14 *CYP2C9* and *VKORC1* variants along with a small subset of environmental factors account for 45.3% of warfarin dosage variability in the SA population. I observed an increase in the number of poor pregnancy outcomes in patients on high doses of warfarin. These results allow us to predict the maintenance dose of warfarin in SA black patients better, thereby reducing the risk of adverse effects, and identify those at risk of having a poor pregnancy outcome.

ACKNOWLEDGEMENTS

I would like to thank everybody that has supported me and aided me in my studies. However, special thanks go to:

- God, thank you for the opportunity and ability to further my studies.

- My supervisor, Professor Amanda Krause. Thank you for all the constructive criticism, time, effort and expertise you have put into helping me with this project.

- Dr Nerine Gregersen for your work leading to this project and for your assistance in the analysis of my results.

- Dr Lize Van Der Merwe advice on and generation of statistical models.

- Dr Tony Lane for assistance with the statistics

- My family and boyfriend. Thank you once again for all your support and patience with me throughout my academic journey. Most of what I have achieved would not have been possible without your support.

TABLE OF CONTENTS

DECLARATION ..... **II**

DEDICATION ..... **III**

PRESENTATIONS ..... **IV**

ABSTRACT ..... **V**

ACKNOWLEDGEMENTS ..... **VI**

TABLE OF CONTENTS ..... **VII**

LIST OF FIGURES ..... **XIII**

LIST OF TABLES ..... **XV**

ABBREVIATIONS ..... **XXV**

1 INTRODUCTION **1**

1.1 PHARMACOGENETICS/GENOMICS 2

1.2 WARFARIN 4

1.2.1 Mode of Action *5*

1.2.1.1 Pharmacokinetics 6

1.2.1.2 Pharmacodynamics 6

1.2.2 Warfarin Administration *6*

1.2.2.1 Environmental Factors 8

1.2.2.2 Genetic Variation 9

1.2.2.2.1 Biotransformation of Warfarin 10

1.2.2.2.2 Biotransformation of Vitamin K 11

1.3 CYTOCHROME P450 2C9 (*CYP2C9)* 13

1.4 VITAMIN K EPOXIDE REDUCTASE COMPLEX SUBUNIT 1 (*VKORC1)* 16

1.5 INDIVIDUALISED WARFARIN THERAPY – CURRENT STATUS 17

1.6 FETAL WARFARIN SYNDROME (FWS) 19

1.6.1 Incidence *20*

1.6.2 Alternative Anticoagulant and Current Regimens *21*

1.7 AIM AND IMPACT OF THIS STUDY 22

2 SUBJECTS, MATERIALS AND METHODS 25

2.1 SUBJECTS 25

2.1.1 Patients *25*

2.1.2 Controls *27*

2.2 MATERIALS AND METHODS 28

2.2.1 DNA Extraction *28*

2.2.2 Polymerase Chain Reaction *29*

2.2.2.1 Primer Sequences 29

2.2.2.2 PCR Conditions 30

2.2.3 Detection on Agarose Gels *31*

2.2.4 Restriction Digests *31*

2.2.5 Sequencing *33*

2.2.5.1 PCR Clean-up 34

2.2.5.2 Cycle Sequencing 34

2.2.5.3 Cycle Sequence Clean-up 35

2.2.5.4 Genetic Analyser 35

2.2.5.4.1 Starting a Sequencing Run 36

2.2.5.4.2 Retrieving a Sequencing Run 36

2.2.6 Analysis of DNA Sequences *37*

2.2.6.1 Novel Variants 37

2.2.7 Statistics *38*

2.2.7.1 Variant Analysis 38

2.2.7.2 Warfarin Dosage Variability Analysis 39

2.2.7.2.1 Environmental Factors 40

2.2.7.2.2 CYP2C9 and VKORC2 Variants 40

2.2.7.3 Pregnancy Outcome Analysis 42

2.2.7.3.1 Environmental Factors 42

2.2.7.3.2 CYP2C9 and VKORC1 Variants 42

3 RESULTS 44

3.1 VARIANT ANALYSIS 44

3.1.1 CYP2C9 Variants *45*

3.1.1.1 Previously Described Variants 45

3.1.1.2 Novel Variants 46

3.1.1.2.1 Coding Sequence Variants 46

3.1.1.2.2 Non-Coding Sequence Variants 48

3.1.2 VKORC1 Variants *51*

3.1.2.1 Previously Described Variants 51

3.1.3 Comparison of Genotype and Allele frequencies of the 14 CYP2C9 and VKORC1 variants in the patient and control samples *53*

3.1.4 Hardy-Weinberg Equilibrium (HWE) *53*

3.1.5 Linkage Disequilibrium (LD) *54*

3.2 WARFARIN DOSAGE VARIABILITY ANALYSIS 56

3.2.1 Environmental Factors *58*

3.2.1.1 Age 58
3.2.1.2 Concomitant Medication 58
3.2.1.2.1 Box Plots 59
3.2.1.2.2 Wilcoxon Test 61
3.2.2 CYP2C9 and VKORC1 variants 62
3.2.2.1 Kruskal-Wallis Test 62
3.2.2.2 Box Plots 63
3.2.2.3 Bar Graphs 65
3.2.2.4 Linear Models 69
3.2.2.5 Haplo.stats Analysis 71
3.2.2.6 Summary of the influence of the 14 variants on warfarin dosage 73
3.3 PREGNANCY OUTCOME ANALYSIS 74
3.3.1 Environmental Factors 76
3.3.1.1 Age 76
3.3.1.2 Heparin 77
3.3.1.3 Warfarin 77
3.3.2 CYP2C9 and VKORC1 variants 79
3.3.2.1 Bar Graphs 80
3.3.2.2 Generalised Linear Models 80
3.3.2.3 Interaction Models 81
3.3.2.4 Haplo.stats Analysis 82
3.3.2.5 Summary of the influence of the 14 variants on pregnancy outcome 83
4 DISCUSSION 85
4.1 VARIANT ANALYSIS 85
4.1.1 CYP2C9 Variants 85

4.1.1.1 Previously Described Variants 85

4.1.1.2 Novel Variants 87

4.1.2 VKORC1 Variants 89

4.1.3 Genetic variation amongst populations of African origin 90

4.1.4 Future Studies involving the observed CYP2C9 and VKORC1 variants 91

4.2 WARFARIN DOSAGE VARIABILITY ANALYSIS 92

4.2.1 Influence of the environmental factors on warfarin dosage 93

4.2.1.1 Age 93

4.2.1.2 Concomitant Medication 93

4.2.2 Influence of 14 CYP2C9 and VKORC1 variants on warfarin dosage 95

4.2.3 Pharmacogenomic testing in SA 97

4.3 PREGNANCY OUTCOME ANALYSIS 100

4.3.1 The influence of the environmental factors on pregnancy outcome 100

4.3.1.1 Age and Heparin 100

4.3.1.2 Warfarin 101

4.3.2 The influence of the 14 CYP2C9 and VKORC1 on pregnancy outcome 102

4.3.3 Genetic counselling for pregnant women on warfarin 104

4.4 LIMITATIONS 105

5 CONCLUSION 108

APPENDIX A 114

APPENDIX B 118

APPENDIX C 119

APPENDIX D 121

APPENDIX E 122

APPENDIX F 123

APPENDIX G 124

APPENDIX H 125

APPENDIX I 126

APPENDIX J 127

APPENDIX K 132

APPENDIX L 151

APPENDIX M 171

APPENDIX N 223

6 REFERENCES 230

LIST OF FIGURES

Figure 1.1: Illustration of interactions between the 30 described genes said to be involved in the mode of action of warfarin (Wadelius and Pirmohammed, 2007) 13

Figure 2.1: Representation of a 3% agarose gel with the different genotypes for the *CYP2C9*2* variant after digestion with *Ava*II 32

Figure 2.2: Representation of a 3% agarose gel with the different genotypes for the *CYP2C9*5* variant after digestion with *Alu*II 33

Figure 3.1: Isoleucine changed to a Valine (I42V, I74V), (Mathews et al., 2000) 47

Figure 3.2: Valine changed to a Glutamine (V76Q), (Mathews et al., 2000) 47

Figure 3.3: Isoleucine change to Threonine (I327T), (Mathews et al., 2000) 47

Figure 3.4: Linkage Disequilibrium Heatmap for the control samples 54

Figure 3.5: Linkage Disequilibrium Heatmap for the patient samples 55

Figure 3.6: Linkage Disequilibrium Heatmap for both the patient and control samples 55

Figure 3.7: Distribution of warfarin dosage (in mg/week) in the patients (n = 110) 57

Figure 3.8: Age distribution amongst the patients (n = 111) 58

Figure 3.9: Box plots depicting the relationship between the concomitant drugs and warfarin dosage 60

Figure 3.10: Box plots representing the influence of variants1 – 8 on warfarin dosage 64

Figure 3.11: Box plots representing the influence of variants 9 – 14 on warfarin dosage .. 64

Figure 3.12: Representation of the distribution of the variant alleles for variants 1 – 4 in each of the dosage groups 66

Figure 3.13: Representation of the distribution of the variant alleles for variants 5 – 8 in each of the dosage groups 66

Figure 3.14: Representation of the distribution of the variant alleles for variants 9 – 12 in each of the dosage groups..... 67

Figure 3.15: Representation of the distribution of the variant alleles for variants 13 and 14 in each of the dosage groups 67

Figure 3.16: Illustration of the number of poor and normal pregnancy outcomes after the patients' 1st three pregnancies on warfarin..... 75

Figure 3.17: Illustration of the effect of maternal age on pregnancy outcome 76

Figure 3.18: Influence of warfarin dosage on pregnancy outcome after one pregnancy 78

Figure 3.19: Influence of warfarin dosage on pregnancy outcome after two pregnancies.. 78

Figure 3.20: Influence of warfarin dosage on pregnancy outcome after three pregnancies 79

Figure 5.1: Bar Graphs for variants 1 – 8 showing their influence on pregnancy outcome 151

Figure 5.2: Bar Graphs for variants 9 – 14 showing their influence on pregnancy outcome 152

Figure 5.3: Picture of an agarose gel run with *CYP2C9* exon 6 fragments..... 223

Figure 5.4: Picture of an agarose gel run with *CYP2C9* exon 8 fragments..... 223

Figure 5.5: Picture of an agarose gel run with *VKORC1* exon 3 fragments..... 224

Figure 5.6: Electropherogram of *VKORC1* exon 1 in control sample 1..... 225

Figure 5.7: Electropherogram of *VKORC1* exon 1 in control sample 1, continued..... 226

Figure 5.8: Electropherogram of *CYP2C9* exon 3, patient 4..... 227

Figure 5.9: Electropherogram of *CYP2C9* exon 3, patient 4, continued..... 228

Figure 5.10: Electropherogram of *CYP2C9* exon 3, patient 4, continued..... 229

LIST OF TABLES

Table 1.1: List of the genes implicated in the action of warfarin to date (Wadelius and Pirmohammed, 2007) 10

Table 1.2: List of *CYP2C9* described variants 15

Table 1.3: List of *VKORC1* described variants 17

Table 2.1: Number of pregnancies amongst the patients..... 26

Table 2.2: Summary of pregnancy information obtained for the patients (n=111)..... 26

Table 2.3: Summary of the number of patient samples and the analyses in which they were used in this study 27

Table 2.4: Summary of the number of control samples and the analyses in which they were used in this study 28

Table 3.1: Genotype and allele frequencies of the previously described *CYP2C9* variants observed in the patient and control samples..... 45

Table 3.2: Description of all the novel *CYP2C9* silent and missense mutations observed within the patient and control samples 46

Table 3.3: Genotype and Allele frequencies of the four novel silent and four novel missense mutations observed in the patient and control samples..... 48

Table 3.4: Summary of the Splice Site predictor website searches for the nine possible splice site mutations 49

Table 3.5: Genotype and Allele frequencies of the nine novel possible splice site mutations observed in the patient and control samples..... 50

Table 3.6: Genotype and Allele frequencies of the ten novel variants, whose effect on Cytochrome P450 is unknown, observed in the patient and control samples 51

Table 3.7: Genotype and Allele frequencies of the previously described *VKORC1* variants observed in the patient and control samples 52

Table 3.8: List of the previously described and novel *CYP2C9* and *VKORC1* variants that were used for comparison and correlation analyses 53

Table 3.9: Description of the three dosage groups and the number of patients in each group 57

Table 3.10: List and frequencies of the concomitant medications taken by the patients 59

Table 3.11: Summary of the results of the Wilcoxon test and box plots depicting the relationship between the concomitant drugs and warfarin dosage 61

Table 3.12: List of analyses used to determine the influence of the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage 62

Table 3.13: List of the three possible genotypes for the 14 *CYP2C9* and *VKORC1* variants 63

Table 3.14: A description of the impact of the two variant genotypes for the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage, based on the box plots 65

Table 3.15: Summary of the results shown in the allele frequency graphs for each of the 14 *CYP2C9* and *VKORC1* variants 68

Table 3.16: Summary of the linear model results for all 14 *CYP2C9* and *VKORC1* variants and environmental factors 70

Table 3.17: List of the allele combinations that significantly influence warfarin dosage in the patients, based on the haplo.stats results 72

Table 3.18: Summary of the results of the different analyses used to determine the influence of the different *CYP2C9* and *VKORC1* variants on warfarin dosage 73

Table 3.19: Summary of all of the analyses used to determine the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome 80

Table 3.20: Summary of the effects of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome according to the generalised linear models 81

Table 3.21: Summary of the effects of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome according to the interaction models 82

Table 3.22: Summary of the number of 1st pregnancies used for the haplo.stats analyses 82

Table 3.23: Summary of the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome 84

Table 4.1: List and allele frequencies of the previously described *CYP2C9* variants that were observed in the SA population 86

Table 4.2: List of the 27 novel *CYP2C9* variants and their affect on mephenytoin 4-hydroxylase 88

Table 4.3: List of three observed *VKORC1* variants and their allele frequencies as reported and observed in the patient and control samples 90

Table 4.4: Summary of the influence of the lasix, slow K, beta blockers and aspirin on warfarin dosage based on the box plots, Wilcoxon test and linear models 94

Table 4.5: Summary of the hypothesised influence of the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage 95

Table 4.6: Summary of the influence of the 14 variants on warfarin dosage and their expected and observed influence on pregnancy outcome 103

Table 5.1: Primer sets for both *CYP2C9* and *VKORC1* gene amplicons 119

Table 5.2: 10x dNTP Mix 119

Table 5.3: PCR Mix 120

Table 5.4: PCR Conditions for all *CYP2C9* and *VKORC1* amplicons 120

Table 5.5: *Ava*II Digest for *CYP2C9*2* 121

Table 5.6: *Alu*II Digest for *CYP2C9*5* 121

Table 5.7: Sequencing Reaction Setup (1/8x) 123

Table 5.8: Exact test for Hardy-Weinberg Equilibrium for all sample groups 127

Table 5.9: P-values of Linkage Disequilibrium for Control Samples 128

Table 5.10: P-values of Linkage Disequilibrium for Patient Samples 129

Table 5.11: P-values of Linkage Disequilibrium for both Control and Patient Samples .. 130

Table 5.12: P-values from the Fisher's Exact and Cochran/Armitage tests 131

Table 5.13: P-values of the influence of the concomitant drugs on warfarin dosage based on the Wilcoxon test 132

Table 5.14: P-values indicating the influence of the 14 *CYP2C9* and *VKORC1* variants based on the Kruskal-Wallis test 132

Table 5.15: Results of the linear model showing the influence of the four concomitant medications on warfarin dosage 133

Table 5.16: Results of the linear model showing the influence pact of the four concomitant medications and age on warfarin dosage 134

Table 5.17: Results of the linear model showing the influence of variant 1 on warfarin dosage accounting for the four concomitant medications and age 134

Table 5.18: Results of the linear model showing the influence of variant 2 on warfarin dosage accounting for the four concomitant medications and age 135

Table 5.19: Results of the linear model showing the influence of variant 3 on warfarin dosage accounting for the four concomitant medications and age 135

Table 5.20: Results of the linear model showing the influence of variant four genotypes on warfarin dosage accounting for the four concomitant medications and age 136

Table 5.21: Results of the linear model showing the influence of variant 5 on warfarin dosage accounting for the four concomitant medications and age 136

Table 5.22: Results of the linear model showing the influence of variant 6 on warfarin dosage accounting for the four concomitant medications and age 137

Table 5.23: Results of the linear model showing the influence of variant 7 on warfarin dosage accounting for the four concomitant medications and age 137

Table 5.24: Results of the linear model showing the influence of variant 8 on warfarin dosage accounting for the four concomitant medications and age 138

Table 5.25: Results of the linear model showing the influence of variant 9 on warfarin dosage accounting for the four concomitant medications and age 138

Table 5.26: Results of the linear model showing the influence of variant 10 on warfarin dosage accounting for the four concomitant medications and age 139

Table 5.27: Results of the linear model showing the influence of variant 11 on warfarin dosage accounting for the four concomitant medications and age 139

Table 5.28: Results of the linear model showing the influence of variant 12 on warfarin dosage accounting for the four concomitant medications and age 140

Table 5.29: Results of the linear model showing the influence of variant 13 on warfarin dosage accounting for the four concomitant medications and age 140

Table 5.30: Results of the linear model showing the influence of variant 14 on warfarin dosage accounting for the four concomitant medications and age 141

Table 5.31: Results of the linear model showing the influence of all the *CYP2C9* variants (1-12) on warfarin dosage accounting for the four concomitant medications and age 142

Table 5.32: Results of the linear model showing the influence of both *VKORC1* variants (13-14) on warfarin dosage accounting for the four concomitant medications and age 143

Table 5.33: Results of the linear model showing the influence of all 14 variants on warfarin dosage accounting for the four concomitant medications and age 144
Table 5.34: Haplo.stats results using all 12 *CYP2C9* variants 145
Table 5.35: Haplo.stats results using *CYP2C9* variants 1 – 6 146
Table 5.36: Haplo.stats results using *CYP2C9* variants 2 – 7 146
Table 5.37: Haplo.stats results using *CYP2C9* variants 3 – 8 147
Table 5.38: Haplo.stats results using *CYP2C9* variants 4 – 9 147
Table 5.39: Haplo.stats results using *CYP2C9* variants 5 – 10 148
Table 5.40: Haplo.stats results using *CYP2C9* variants 6 – 11 148
Table 5.41: Haplo.stats results using *CYP2C9* variants 7 – 12 149
Table 5.42: Haplo.stats results using *VKORC1* variants 13 and 14 149
Table 5.43: Haplo.stats results using 13 of the *CYP2C9* and *VKORC1* variants* 150
Table 5.44: Generalised linear model result for age and number of pregnancies 153
Table 5.45: Generalised linear model result for age, number of pregnancies, warfarin and heparin 153
Table 5.46: Results of the generalised linear model showing the influence of variant 1 on pregnancy outcome 153
Table 5.47: Results of the generalised linear model showing the influence of variant 2 on pregnancy outcome 154
Table 5.48: Results of the generalised linear model showing the influence of variant 3 on pregnancy outcome 154
Table 5.49: Results of the generalised linear model showing the influence of variant 4 on pregnancy outcome 154
Table 5.50: Results of the generalised linear model showing the influence of variant 5 on pregnancy outcome 155

Table 5.51: Results of the generalised linear model showing the influence of variant 6 on pregnancy outcome 155

Table 5.52: Results of the generalised linear model showing the influence of variant 7 on pregnancy outcome 155

Table 5.53: Results of the generalised linear model showing the influence of variant 8 on pregnancy outcome 156

Table 5.54: Results of the generalised linear model showing the influence of variant 9 on pregnancy outcome 156

Table 5.55: Results of the generalised linear model showing the influence of variant 10 on pregnancy outcome 156

Table 5.56: Results of the generalised linear model showing the influence of variant 11 on pregnancy outcome 157

Table 5.57: Results of the generalised linear model showing the influence of variant 12 on pregnancy outcome 157

Table 5.58: Results of the generalised linear model showing the influence of variant 13 on pregnancy outcome 157

Table 5.59: Results of the generalised linear model showing the influence of variant 14 on pregnancy outcome 158

Table 5.60: Interaction model showing the influence of variant 1 on pregnancy outcome 159

Table 5.61: Interaction model showing the influence of variant 2 on pregnancy outcome 159

Table 5.62: Interaction model showing the influence of variant 3 on pregnancy outcome 160

Table 5.63: Interaction model showing the influence of variant 4 on pregnancy outcome 160

Table 5.64: Interaction model showing the influence of variant 5 on pregnancy outcome 161

Table 5.65: Interaction model showing the influence of variant 7 on pregnancy outcome 161

Table 5.66: Interaction model showing the influence of variant 8 on pregnancy outcome 162

Table 5.67: Interaction model showing the influence of variant 12 on pregnancy outcome 162

Table 5.68: Interaction model showing the influence of variant 14 on pregnancy outcome 163

Table 5.69: Haplo.stats result using variants 1-4 in pregnancies on warfarin 164

Table 5.70: Haplo.stats result using variants 1-4 in pregnancies off warfarin 164

Table 5.71: Haplo.stats result using variants 2-5 in pregnancies on warfarin 165

Table 5.72: Haplo.stats result using variants 2-5 in pregnancies off warfarin 165

Table 5.73: Haplo.stats result using variants 3-6 in pregnancies on warfarin 165

Table 5.74: Haplo.stats result using variants 3-6 in pregnancies off warfarin 166

Table 5.75: Haplo.stats result using variants 4-7 in pregnancies on warfarin 166

Table 5.76: Haplo.stats result using variants 4-7 in pregnancies off warfarin 166

Table 5.77: Haplo.stats result using variants 5-8 in pregnancies on warfarin 167

Table 5.78: Haplo.stats result using variants 5-8 in pregnancies off warfarin 167

Table 5.79: Haplo.stats result using variants 6-9 in pregnancies on warfarin 167

Table 5.80: Haplo.stats result using variants 6-9 in pregnancies off warfarin 168

Table 5.81: Haplo.stats result using variants 7-10 in pregnancies on warfarin 168

Table 5.82: Haplo.stats result using variants 7-10 in pregnancies off warfarin 168

Table 5.83: Haplo.stats result using variants 8-11 in pregnancies on warfarin..... 169

Table 5.84: Haplo.stats result using variants 8-11 in pregnancies off warfarin 169

Table 5.85: Haplo.stats result using variants 9-12 in pregnancies on warfarin..... 169

Table 5.86: Haplo.stats result using variants 9-12 in pregnancies off warfarin 170

Table 5.87: Haplo.stats result using variants 13-14 in pregnancies on warfarin..... 170

Table 5.88: Haplo.stats result using variants 13-14 in pregnancies off warfarin 170

Table 5.89: Genotype Data for the first 15 previously described CYP2C9 variants in the 100 Control Samples 171

Table 5.90: Genotype Data for variants 16 – 30 of the previously described CYP2C9 variants in the 100 Control Samples..... 175

Table 5.91: Genotype Data for the first 13 new CYP2C9 variants observed in the 100 Control Samples 178

Table 5.92: Genotype Data for the new CYP2C9 variants observed in the Exon 7, 8 and 9 fragments in the 100 Control Samples 182

Table 5.93: Genotype Data for the first 15 previously described CYP2C9 variants observed in the 113 Patient Samples 185

Table 5.94: Genotype Data for the previously described CYP2C9 variants 16 – 30, observed in the 113 Patient Samples..... 189

Table 5.95: Genotype Data for the first 13 new CYP2C9 variants observed in the 113 Patient Samples 194

Table 5.96: Genotype Data for the new CYP2C9 variants observed in the Exon 7, 8 and 9 fragments of the 113 Patient Samples 198

Table 5.96: Genotype Data for the previously described VKORC1 variants observed in the 100 Control Samples 202

Table 5.97: Genotype Data for the previously described VKORC1 variants observed in the 113 Patient Samples 205

Table 5.98: Clinical Information collected from the 113 Patient Samples 210

Table 5.99: Concomitant Medication information collected from the 113 Patient Samples 214

Table 5.100: Pregnancy information collected from the 113 Patient Samples 218

ABBREVIATIONS

A	Adenine
bp	Base pairs
BDT	Big Dye terminator ready reaction mix
C	Cytosine
cm	Centimetre
CHB	Chris-Hani Baragwanath Hospital
d.f	Degrees of freedom
ddH_2O	Double distilled water (Autoclaved distilled water)
dH_2O	Distilled water
DNA	Deoxyribose nucleic acid
ddNTP	dideoxyribonucleoside triphosphate
dNTP	deoxyribonucleoside triphosphate
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium Bromide
FDA	Food and Drug Association
G	Guanine
g	Gram
HWE	Hardy-Weinberg Equilibrium
INR	International Normalisation Ratio
kb	Kilo bases
$MgCl_2$	Magnesium Chloride
mg	Milligrams
mM	Milli Molar
µl	Micro litres
ng	Nanograms
NHLS	National Health Laboratory Service
PCR	Polymerase Chain Reaction
pM	Pico Molar
PT	Prothrombin time
RFLP	Restriction Fragment Length Polymorphism
SA	South Africa
SNP	Single nucleotide polymorphisms
T	Thymine
Taq	DNA polymerase isolated from the bacterium *Thermus aquaticus*
TBE	Tris Borate EDTA
TE	Tris-EDTA
TEC	Thromboembolic Complications
USA	United States of America
UV	Ultra Violet light

1 INTRODUCTION

Science fiction: A genera intended to dumbfound and inspire its audience, has shown itself to be closer to non-fiction than its authors have implied. The explosion of modern technology has given us the tools and skills for the discovery and understanding of life, from outer-space to the depths of the ocean, all in record time. Perhaps the most fundamental of all questions is the amount of time one has to live and how we can prolong that? It is this question that is often the driving force behind modern medicine. We use fast, efficient tests to determine the cause of a particular disease, syndrome or infection, with the goal of administering the most effective treatment available. We are also using modern technology to improve our understanding of the human body and how it interacts with itself and its external environment.

Often, however, with the accumulation of knowledge one realises how little understanding we really have. Human medical genetics, as an example, in the 1950's was primarily focused on diseases caused by single defective genes that could be traced back through families, and disorders due to defects in the structure or number of chromosomes (The Royal Society, 2005). Further studies in this field revealed that not all inherited disorders were a result of a single defective gene but some as a result of the interaction between multiple genes and the environment (The Royal Society, 2005). The completion of the human genome project has revealed far more complexities in our genome than previously thought (The Royal Society, 2005); providing information on how individual genes function and are regulated; biological processes; used as a framework for developing new therapies and as a wide-scale application for mutation screening in the hope of shifting medical care from treating diseases, to preventing diseases (Strachan and Read, 2004).

1.1 Pharmacogenetics/genomics

The term pharmacogenetics was coined and published in 1959 by Friedrich Vogel, and refers to the study of single genes that modify drug action (Kalow, 2005; The Royal Society, 2005). This field was initiated by three independent discoveries, all of which showed inter-individual differences in drug response (The Royal Society, 2005). The first of these discoveries was in African-American soldiers who developed severe anaemia, after taking the anti-malaria drug primaquine, due to a deficiency in the enzyme glucose-6-phosphate-dehydrogenase (Reviewed in: The Royal Society, 2005). The second discovery was the identification of slow and rapid metabolisers of isoniazid, a drug used for the treatment of tuberculosis (Reviewed in: The Royal Society, 2005). The third discovery was the identification of patients who showed prolonged effects of the anaesthetic agent succinycholine (Reviewed in: The Royal Society, 2005).

Such individual variation creates huge clinical challenges, accounting for 106 000 patient deaths and 2.2 million injuries due to adverse reactions to prescribed drugs in the USA and about one in 15 hospital admissions in the UK annually (Wolf et al., 2000). Research in pharmacogenetics is focused in two main directions: identifying specific genes and gene products associated with various diseases, which may act as targets for new drugs, and identifying genes and allelic variants in genes that affect individual responses to currently available drugs (Wolf et al., 2000; Ensom et al., 2001). These studies all investigate pharmacological consequences of single gene variations.

Numerous studies have shown that most differences in drug response are not due to mutations in a single gene but the altered function of numerous genes interacting with environmental factors, making drug response multifactorial (Kalow, 2005). It was this

discovery that resulted in the birth of pharmacogenomics, made possible by the development of high-throughput technology capable of investigating the structure and expression of entire genomes (Ensom et al., 2001; Kalow, 2005). Pharmacogenomics, by definition, is a biotechnological science that combines the techniques of medicine, pharmacology, and genomics (many genes and their function) and is concerned with developing drug therapies to compensate for genetic differences in patients which cause varied responses to a single therapeutic regimen (Merriam-Webster's Medical Dictionary).

Gene expression is variable, altered by factors such as other gene expression interactions, epigenetic changes or environmental factors, including sleep, emotions, exercise, diet, age, sex, co-morbidity and drugs (Kalow, 2005). Drug addictions may be explained through the increased expression of the gene that is responsible for metabolising a particular drug, due to regular intake of the drug (Kalow, 2005). When dealing with multifactorial disorders it is not uncommon to find similar-looking diseases in patients that are caused by different genes and thus may require different drug therapies to combat these diseases (Kalow, 2005).

Personalised medicine refers to the use of a patient's genetic make-up and other environmental factors to predict the most effective drug therapy for that patient, reducing their risk of adverse effects (Reviewed in: The Royal Society, 2005). However, this is not an easy task because of the complexity of the interactions involved in drug treatment. Nevertheless, advances are being made and it is already evident that polymorphisms in any one of many genes that encode drug receptors, drug transporters, cell signalling pathways and those involved in drug metabolism and disposition can account for a large proportion of drug response variability (Wolf et al., 2000; Yin et al., 2007). For example, adverse

effects to drugs known to be metabolised by a genetically variable enzyme may be avoided by pre-testing the patient for genetic variants within that gene and administering the drug to those patients whose enzymatic levels are normal or by altering the dosage according to their metabolic state (Kalow, 2005; Yin et al., 2007). This pre-testing is usually limited to drugs that are potentially toxic to a level higher than the average drug (Kalow, 2005; Yin et al., 2007). Personalised medicine would be drastically improved if one could obtain a complete genetic and environmental profile of the patient. This is not yet economically and practically viable but one can use genetic variation amongst different populations as a predictor. Metabolising enzymes, as an example, show significant variation between populations and thus result in altered drug response (Kalow, 2005; Yin et al., 2007). While there is already clinical application to the use of genetic information in drug administration, substantial improvements can be expected within the next decade, paralleled by the improvement in technology. This project involves the study of two genes and a small subset of environmental factors that influence the action of warfarin in black South African women.

1.2 Warfarin

In the early 1920s farmers in the northern United States and Canada noticed that their cattle were dying of uncontrollable bleeding from minor injuries or due to internal haemorrhage (Schofield, 1924). It was only in 1929 that Dr Roderick established that the deaths in the cattle were due to a lack of functioning prothrombin, as a result of ingesting mouldy silage made from sweet clover that acted as an anticoagulant (Roderick, 1931). In 1940, chemists Karl Paul Link and his student Harold Campbell from the University of Wisconsin determined that this anticoagulant substance, isolated from the mouldy sweet clover, was a coumarin derivative: 4-hydroxycoumarin (Stahmann, 1941; Kresge et al.,

2005). Based on this discovery chemists began developing more potent coumarin-based anticoagulants for use as rodent poisons. This resulted in the discovery of warfarin in 1948 and its registration as a rodenticide in the USA in 1952 (O'Reilly et al., 1963; Kresge et al., 2005).

Warfarin was later studied for its use as a therapeutic anticoagulant as a result of a botched suicide attempt by a US naval officer, who ingested warfarin but recovered fully (O'Reilly et al., 1963). Warfarin was approved for medical use in humans in 1954 and is the gold standard for the long-term prevention of thromboembolism world-wide (O'Reilly et al., 1963; Shapiro, 2003; Greaves, 2005). In the USA it is the most frequently prescribed oral anticoagulant, the fourth most prescribed cardiovascular agent and the eleventh most prescribed drug overall (Horton and Bushwick, 1999; Rettie et al., 2006). In the UK it is estimated that over one million people take warfarin (Greaves, 2005). Despite advances in the development of novel, alternative oral anticoagulants warfarin is likely to be used widely for at least the next decade (Greaves, 2005; Rettie et al., 2006).

1.2.1 Mode of Action

Warfarin causes anticoagulation by inhibiting vitamin K epoxide reductase, an enzyme responsible for the recycling of vitamin K (Greaves, 2005; Rettie et al., 2006). Vitamin K is essential for the post-translational carboxylation of glutamate residues on proteins dependent on vitamin K. Vitamin K dependent proteins include coagulation factors II (prothrombin), VII, IX and X, and endogenous anticoagulant proteins C and S (Horton and Bushwick, 1999; Greaves, 2005; Yin et al., 2007). Therapeutic doses of warfarin reduce the production of functional vitamin K dependent clotting factors by 30-50% and decrease

the activity of secreted clotting factors by 10-40%, rendering the coagulation system functionally deficient (Horton and Bushwick, 1999).

1.2.1.1 Pharmacokinetics

Warfarin is a racemic mixture of stereoisomers, which are 99% bound to albumin and alpha-1-acid glycoproteins (Horton et al., 1999; Wadelius and Pirmohammed, 2007). It is metabolised in the liver and kidneys, with subsequent excretion of its inactive metabolites through urine and stools (Horton and Bushwick, 1999).

1.2.1.2 Pharmacodynamics

The anticoagulant activity of warfarin depends on the clearance of functional clotting factors from the systemic circulation after administration. This is dependent on the half-lives of the clotting factors (Horton and Bushwick, 1999). The antithrombotic (inability to expand or form clots) effect of warfarin depends on the clearance of functional factor II (prothrombin), which has a half-life of 50 hours in patients with normal hepatic function, and thus may take up to five days to achieve (Horton and Bushwick, 1999).

1.2.2 Warfarin Administration

The aim of anticoagulant therapy is to administer the lowest possible dose of the anticoagulant to prevent clot formation or expansion (Horton and Bushwick, 1999). The dosage of warfarin administered to a patient is monitored using a method known as the International Normalisation Ratio (INR), which measures the anticoagulant effect of warfarin based on prothrombin time (PT) (Greaves, 2005). In most cases the target INR is 2.5 with a range of 2.0-3.0, which is associated with an optimal relationship between antithrombotic efficacy and bleeding risk (Greaves, 2005). However, even with the best

possible management, patients are within the target INR range for only 50-70% of the time, on average (Greaves, 2005). As with most drugs, side-effects are often a problem. The most common side-effects associated with warfarin treatment are haemorrhagic complications and thrombosis. Major and fatal bleeding events occur at a rate of 7.2 and 1.3/100 patient years, respectively, and are most likely to occur within the first 90 days of therapy (Wadelius and Pirmohammed, 2007). The risk of a bleeding episode is higher when the INR is above 3.0, but also occurs within the therapeutic range (Wadelius and Pirmohammed, 2007). A maintenance dose of warfarin is said to be the dosage required to maintain the patient's INR within the therapeutic range. Maintenance doses of warfarin may range between 1-10mg/day, with an average maintenance dose between four and 6mg/day (Horton et al., 1999, Greaves, 2005). The standard initiation dose of warfarin is 5mg/day, which is adjusted according to the patient's INR readings, to obtain an adequate maintenance dose (Hillman et al., 2005). However, the earliest change in the INR occurs only 24-36 hours after administration of the first dose and maximum anticoagulant effect is only achieved 72-96 hours after administration (Horton et al., 1999). The average time it takes to determine an appropriate maintenance dosage for a patient is approximately one month, during which time the patient has an increased risk of both thrombotic events and bleeding episodes (Next Generation Pharmaceutical Website; Rettie et al., 2006).

The risk of thrombotic events and bleeding, the drug's narrow therapeutic range and lag time, along with interindividual differences in drug response all make warfarin a difficult drug to administer (Rettie et al., 2006; Wadelius and Pirmohammed, 2007). Warfarin dose requirements, stability of anticoagulation and risk of bleeding are influenced by environmental factors and genetic variation in genes that alter the action of the drug (reviewed in: Rettie et al., 2006; Wadelius and Pirmohammed, 2007). Despite its

complications, warfarin remains the gold standard for the prevention of thromboembolic events as it has been shown to prevent 20 strokes for every bleeding episode it induces (Horton and Bushwick, 1999; Rettie et al., 2006).

1.2.2.1 Environmental Factors

The environmental factors that influence warfarin dosage include the intake of vitamin K, co-morbidity, age, gender, concurrent medication and body surface area. Warfarin targets vitamin K epoxide reductase, an enzyme essential for the recycling of vitamin K. Therefore, a high intake of fat-soluble vitamin K can reverse the action of warfarin. A low or erratic intake of dietary vitamin K may be partly responsible for the unstable control of anticoagulation (reviewed in: Rettie et al., 2006; Wadelius and Pirmohammed, 2007).

Warfarin dosage has an inverse relationship with age; i.e. older patients require lower doses (Horton and Bushwick, 1999). When comparing maintenance dose between genders, women tend to require lower doses than men (Horton and Bushwick, 1999).

Drug-drug interactions are often a problem during drug treatments. Warfarin is no exception as, in most instances, drug-drug interactions either inhibit or induce warfarin metabolism (Horton and Bushwick, 1999; reviewed in: Rettie et al., 2006). The drugs that pose the most complications are those used for short-term indications, as opposed to drugs administered for long periods, such as those used for chronic diseases, diabetes for example (Horton and Bushwick, 1999). Aspirin, Cordarone, Epanutin and Nifedipine (described in table 3.10, section 3.2.1.2) may decrease warfarin dosage (Rx Drug Index Database; Heart Health Website); while Tegretol may increase warfarin dosage (Horton and Bushwick, 1999).

1.2.2.2 Genetic Variation

It has been estimated that approximately 30 genes may be involved in the mechanism through which warfarin exerts its anticoagulant effect (Wadelius and Pirmohammed, 2007). Biochemical reactions involved in the action of warfarin are: the biotransformation of warfarin (transportation, metabolism, and cytochrome P_{450} inducibility) and biotransformation of vitamin K (transportation, the vitamin K cycle, vitamin K-dependent proteins and other coagulation proteins) (Reviewed in: Wadelius and Pirmohammed, 2007).

Despite the vast number of genes involved in the mode of action of warfarin the *CYP2C9* and *VKORC1* (described in more detail in sections 1.3 and 1.4, respectively) genes are the most important with respect to the pharmacokinetics and pharmacodynamics of warfarin, respectively (Rettie et al., 2006; Wadelius and Pirmohammed, 2007). These two genes, along with a small subset of environmental factors accounts for 50-60% of warfarin dosage variability (Wadelius and Pirmohammed, 2007). *CYP2C9*, *VKORC1*, *PROC*, *EPHX1*, *GGCX*, *ORM1* and *ORM2* genes with age, bodyweight and drug interactions account for 73% of warfarin dosage variability in Caucasians (Wadelius et al., 2007). Table 1.1 summarises the 30 genes implicated in the action of warfarin to date.

Table 1.1: List of the genes implicated in the action of warfarin to date (Wadelius and Pirmohammed, 2007)

Biochemical Reaction		Protein Name	Gene
Biotransformation of warfarin	Transport	Alpha-1-acid glycoprotein 1, Orosomucoid 1	*ORM1*
		Alpha-1-acid glycoprotein 2, Orosomucoid 2	*ORM2*
		P-glycoprotein, Multidrug resistance protein 1	*ABCB1 (MDR1)*
	Metabolism	Cytochrome P450 2C9	*CYP2C9*
		Cytochrome P450 1A1	*CYP1A1*
		Cytochrome P450 1A2	*CYP1A2*
		Cytochrome P450 2A6	*CYP2A6*
		Cytochrome P450 2C8	*CYP2C8*
		Cytochrome P450 2C18	*CYP2C18*
		Cytochrome P450 2C19	*CYP2C19*
		Cytochrome P450 3A4	*CYP3A4*
		Cytochrome P450 3A5	*CYP3A5*
	Cytochrome P_{450} Inducibility	Pregnane X receptor	*NR1I2*
		Constitutive androstane receptor	*NR1I3*
Biotransformation of vitamin K	Transport	Apolipoprotein E	*APOE*
	Vitamin K cycle	Vitamin K epoxide reductase	*VKORC1*
		Epoxide hydrolase 1, microsomal	*EPHX1*
		NAD(P)H dehydrogenase, quinine 1	*NQO1*
		Calumenin	*CALU*
		Gamma-glutamyl carboxylase	*GGCX*
	Vitamin K-dependent proteins	Coagulation factor II, prothrombin	*F2*
		Coagulation factor VII	*F7*
		Coagulation factor IX	*F9*
		Coagulation factor X	*F10*
		Protein C	*PROC*
		Protein S	*PROS1*
		Protein Z	*PROZ*
		Growth-arrest-specific protein	*GAS6*
	Other coagulation proteins	Anti-thrombin III	*SERPINC1*
		Coagulation factor V	*F5*

1.2.2.2.1 Biotransformation of Warfarin

In the circulating blood, warfarin is 99% bound to albumin and alpha-1-acid glycoproteins (Reviewed in: Wadelius and Pirmohammed, 2007). A study carried out by Nakagawa et al. (2003) shows that warfarin preferentially binds to certain genetic variants of alpha-1-acid

glycoproteins, which are encoded by *ORM1* and *ORM2* (orosomucoid gene 1 and 2 respectively). A recent study shows that polymorphisms and different haplotypes of these two genes influence warfarin dose (Wadelius et al., 2007).

Warfarin is administered as a racemate comprising *R*- and *S*-enantiomers, the latter being 3-5 times more active than the former. The *S*-form is metabolised by cytochrome P_{450} *2C9* to an inactive 7-hydroxywarfarin. Polymorphisms in this gene play a significant role in warfarin dosage sensitivity (Rettie et al., 1992; reviewed in: Hirsch et al., 1998; Reviewed in: Wadelius et al., 2007). *S*-warfarin may also be metabolised by other members of the cytochrome P_{450} enzymes, such as CYP2C8, CYP2C18 and CYP2C19 to form 4-hydroxywarfarin (Reviewed in: Wadelius et al., 2007). These are minor pathways and although they show some significance with respect to warfarin dosage, may be explained through linkage disequilibrium with *CYP2C9* (described in section 1.3) (Reviewed in: Wadelius et al., 2007). *R*-warfarin is primarily metabolised by cytochrome P_{450} enzymes CYP1A2, CYP3A4, CYP1A1, CYP2C8, CYP2C18, CYP2C19 and CYP3A5 (reviewed in: Hirsch et al., 1998; Reviewed in: Wadelius et al., 2007). These genes show weak associations with warfarin dosage (Wadelius et al., 2007). The induction of these P450 isoforms is dependent on the nuclear hormone receptors: pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), encoded by the *NR1I2* and *NR1I3* genes, respectively (Reviewed in: Wadelius et al., 2007). Haplotype analysis of the *NR1I2* gene shows some association with warfarin dosage (Wadelius et al., 2007).

1.2.2.2.2 Biotransformation of Vitamin K

Vitamin K is absorbed from the small intestine along with dietary fat. It is transported by chylomicrons in the blood and subsequently cleared by the liver through an APOE

(apolipoprotein E) receptor-specific uptake (Reviewed in: Wadelius and Pirmohammed, 2007). The uptake of vitamin K_1 varies depending on different *APOE* variants. Polymorphisms within *APOE* are significantly associated with warfarin dosage (Wadelius et al., 2007)

Mutations within the vitamin K epoxide reductase gene have been shown to confer warfarin resistance (Rost et al., 2004, Harrington et al., 2005; Rettie et al., 2006). However, one polymorphism in the promoter region of this gene decreases warfarin dosage through a reduction in the VKOR (Vitamin K epoxide reductase) (Reider et al., 2005). It has been suggested that this reductase resides in the endoplasmic reticulum and may be complexed with microsomal epoxide hydrolase (encoded by *EPHX1*). It is this multiprotein complex that is responsible for vitamin K epoxide reduction (Cain et al., 1997, Morisseau and Hammock, 2005). Polymorphisms in the *EPHX1* gene show a significant association with warfarin dose (Wadelius et al., 2007). Nicotine adenine dinucleotide phosphate (NAD(P)H) dehydrogenase, encoded by *NQO1*, has the potential to reduce dietary vitamin K. The endoplasmic reticulum chaperone protein calumenin (encoded by *CALU*) is able to inhibit the vitamin K cycle. Polymorphisms in *CALU*, and not *NQO1*, are associated with warfarin dose (Wadelius et al., 2007).

A very rare autosomal recessive bleeding disorder, caused by mutations in the gamma-glutamyl carboxylase gene (*GGCX*), results in the combined deficiency of the vitamin K-dependent coagulation factors II, VII, IX and X, and proteins C, S and Z (Brenner et al., 1998, Rost et al., 2004). Mutations within this gene are associated with warfarin dose; however, the effect appears to be modest (Wadelius et al., 2007). Similarly, mutations in the genes that encode these vitamin K-dependent factors and proteins may also influence

warfarin dose, but studies have been inconclusive or contradictory (Wadelius and Pirmohammed, 2007). Antithrombin III, a non-vitamin K-dependent protein, inhibits factors II, IX, X, XI and XIII. A deficiency in antithrombin III caused by mutations in its encoding gene *SERPINC1* may create a hypercoagulable state during warfarin induction (Chan et al., 2000, Dahlback, 2005). Figure 1.1 illustrates the interactions of all 30 genes said to be involved in the mode of action of warfarin.

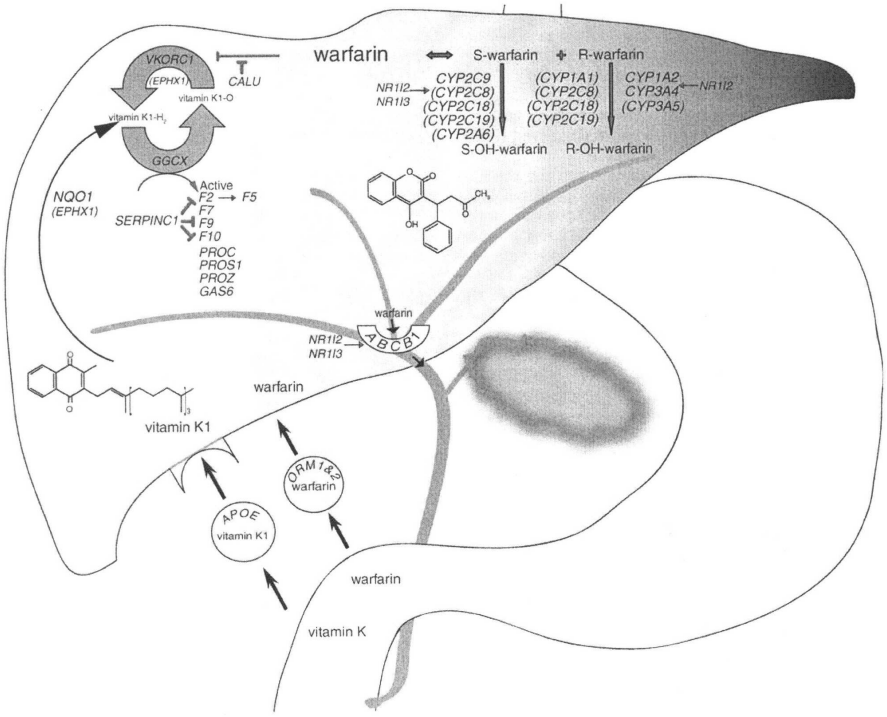


Figure 1.1: Illustration of interactions between the 30 described genes said to be involved in the mode of action of warfarin (Wadelius and Pirmohammed, 2007)

1.3 Cytochrome P450 2C9 (*CYP2C9)*

CYP2C9 is one of approximately 50 major drug-metabolising CYP450 isoforms. It is the second of four *CYP2C* genes (*CYP2C8-CYP2C9-CYP2C19-CYP2C18*) clustered in a

500kb region on 10q24 (Gray et al., 1995; Rettie et al., 2006; Yin et al., 2007). It contains nine exons, translating 490 amino acid residues, which encodes a mephenytoin 4-hydroxylase (GenBank, 2007). This enzyme is responsible for the metabolism of endogenous compounds and xenobiotics, including warfarin (OMIM, 2007). This gene, like many, is polymorphic. Variants within this gene are known to alter warfarin metabolism, resulting in patients requiring altered doses of warfarin to maintain adequate anticoagulation (Allabi et al., 2004; reviewed in: Rettie et al., 2006). To date there are 30 described variants within this gene, 28 of which are missense mutations and two frameshift mutations (*CYP2C9* Allele Nomenclature Database). Like many genetic variants certain variants are more common within certain populations. Table 1.2 describes these different variants, their effect on enzyme activity and the populations in which they have been described. *CYP2C9*1* is the wild type, thereafter the variants are numbered according to the order in which they were identified.

Table 1.2: List of *CYP2C9* described variants

Variant	Nucleotide Change	Exon	Protein Change	Enzyme Activity		Population
				In vivo	*In vitro*	
2	430 C>T	3	R144C	Unknown	Decreased	Caucasian[1, 9]
3	1075 A>C	7	I359L	Decreased	Decreased	Caucasian[1,9]
4	1076 T>C	7	I359T	Unknown	Decreased	Japanese[2, 9]
5	1080 C>G	7	D360E	Decreased	Decreased	African*[, 3, 4, 9]
6	818delA	5	Frameshift	None	Unknown	African*[, 4]
7	55 C>A	1	L19I	Unknown	Unknown	African*[, 5]
8	449 G>A	3	R150H	Decreased	Increased	African*[, 4, 5, 9]
9	752 A>G	5	H251R	Unknown	Unknown	African*[, 5]
10	815 A>G	5	E272G	Unknown	Unknown	Unknown[5]
11	1003 C>T	7	R355W	Decreased	Decreased	Caucasian [4, 9], African*[,4, 5, 9]
12	1465 C>T	9	P489S	Unknown	Decreased	Unknown[5, 9]
13	269 T>C	2	L90P	Decreased	Decreased	Chinese[6, 9]
14	374 G>A	3	R125H	Unknown	Decreased	Indian[7, 9]
15	485 C>A	4	S162X	Unknown	None	Indian[7, 9]
16	895 A>G	6	T299A	Unknown	Decreased	Chinese[7, 9]
17	1144 C>T	7	P382S	Unknown	Decreased	Chinese[7, 9]
18	1190 A>C	8	D397A	Unknown	Decreased	Indian[7]
19	1362 G>C	9	Q454H	Unknown	Decreased	Chinese[7, 9]
20	208 G>C	2	G70R	Unknown	Unknown	Malay[7]
21	89 C>T	1	P30L	Unknown	Unknown	Unknown[8]
22	121 A>G	1	N41D	Unknown	Unknown	Unknown[8]
23	226 G>A	2	V76M	Unknown	Unknown	Unknown[8]
24	1060 G>A	7	E354K	Unknown	Unknown	Unknown[8]
25	353-362 del AGAAATGGAA	3	Frameshift	Unknown	None	Unknown[8]
26	389 C>G	3	T130R	Unknown	Decreased	Unknown[8]
27	449 G>T	3	R150L	Unknown	Unknown	Unknown[8]
28	641 A>T	4	Q214L	Unknown	Decreased	Unknown[8]
29	835 C>A	6	P279T	Unknown	Unknown	Unknown[8]
30	1429 G>A	9	A477T	Unknown	Decreased	Unknown[8]

*African as defined as: African American, African Pygmies or Beninese.
References: [1] Aithal et al., 1999, [2] Imai et al., 2000, [3] Dickmann et al., 2001, [4]Allabi et al., 2003 and 2004, [5] Blaisdell et al., 2004,[6] Si et al., 2004, [7] Zhao et al., 2004, [8] *CYP2C9* Allele Nomenclature Database,
[9] Reviewed in: Yin et al., 2007

The most common variants found in the Caucasian populations are *CYP2C9*2* and *CYP2C9*3*. Both of these variants decrease warfarin metabolic activity drastically by 88% and 95%, respectively (Aithal et al., 1999; reviewed in: Yin et al., 2007). There are approximately six variants (*CYP2C9*5*, *CYP2C9*6*, *CYP2C9*7*, *CYP2C9*8*, *CYP2C9*9* and *CYP2C9*11*) that have been described among populations of African origin. The *CYP2C9*5* variant, first identified in African-American patients by Dickmann et al.,

(2001) shows decreased enzyme activity levels ranging from 8-18%. Variant *CYP2C9*8* decreases warfarin metabolism *in vivo*, but increases warfarin metabolism *in vitro* (*CYP2C9* Allele Nomenclature Database). Variant *CYP2C9*11* decreases warfarin metabolism, but the extent is unknown (*CYP2C9* Allele Nomenclature Database). Variants that are common within populations of Asian origin are *CYP2C9*2, CYP2C9*3, CYP2C9*4* and *CYP2C9*13-20*. Variants *CYP2C9*21-30* have all be described in the *CYP2C9* Allele Nomenclature Database, but their population distributions are unknown. To date no studies determining the frequencies of any of these described variants have been carried out in South African populations.

1.4 Vitamin K Epoxide Reductase Complex Subunit 1 (*VKORC1)*

The *VKORC1* gene, identified in 2004, is located at 16p11.2 (Li et al., 2004). It is 5126bp in length and consists of three exons. It encodes a 163 amino acid transmembrane protein of the endoplasmic reticulum, known as vitamin K epoxide reductase (Rettie et al., 2006; OMIM, 2007). This enzyme is responsible for recycling vitamin K. Recycled vitamin K is necessary for the activation of vitamin K-dependent coagulation factors and certain anticoagulant proteins (OMIM, 2007). Later in 2004, four mutations within this gene were reported to result in warfarin resistance and one to result in vitamin K-dependent clotting factor, deficiency, type 2 (Rost et al., 2004). Since then approximately nine novel variants have been identified within this gene (D'Andrea et al., 2005, Harrington et al., 2005, Reider et al., 2005). Most of these variants are missense mutations, however one polymorphism is found in Intron 1, one in the 3'UTR (D'Andrea et al., 2005) and one in the promoter region of the *VKORC1* gene (Reider et al., 2005). Table 1.3 describes all 13 known *VKORC1* variants and their effects on vitamin K epoxide reductase.

Table 1.3: List of *VKORC1* described variants

Nucleotide Change	Exon	Protein Change	Phenotype	Population
-1639 G>A	Promoter	Decreases gene expression	Decreases level of VKOR and warfarin dosage[4]	Caucasian, Asian, and low in African [4]
1173 C>T	Intron 1	Unknown	Possibly warfarin resistance[2]	Caucasian (Italian) [2]
85 G>T	1	V29L	Warfarin resistance[1]	Caucasian (Lebanese & German) [1]
112 G>T	1	D38Y	None[2]	Caucasian (Italian) [2]
129 C>T	1	C43C	None[2]	Caucasian (Italian) [2]
134 T>C	1	V45A	Warfarin resistance[1]	Caucasian (Lebanese & German) [1]
172 A>G	1	R58G	Warfarin resistance[1]	Caucasian (Lebanese & German) [1]
196 G>A	2	V66M	None[3]	Caucasian [3]
292 C>T	3	R98W	Vitamin K-dependent clotting factor deficiency type II[1]	Caucasian (Lebanese & German) [1]
3462 C>T	3	L120L	None[2]	Caucasian (Italian) [2]
3488 T>G	3	L128R	Warfarin resistance[1]	Caucasian (Lebanese & German) [1]
3556 G>A	3	R151G	None[2]	Caucasian (Italian) [2]
3730 G>A	3'UTR	Unknown	Unknown[2]	Caucasian (Italian) [2]

[1] Rost et al., 2004, [2] D'Andrea et al., 2005, [3] Harrington et al., 2005, [4] Reider et al., 2005.

Patients with variants within this gene usually show some level of warfarin resistance which results in these patients requiring increased doses of warfarin to maintain adequate anticoagulation (D'Andrea et al., 2005). The -1639 G>A promoter variant, however, results in a reduced amount of vitamin K epoxide reductase and therefore a reduction in warfarin dosage (Reider et al., 2005). The frequencies of the described *VKORC1* variants have not been determined in the South African populations, until now.

1.5 Individualised Warfarin Therapy – Current Status

In 2004, Hillman, Wilke and colleagues developed a multivariate warfarin dosing model that incorporates age, body size, co-morbidity (diabetes), clinical indication (valve replacement) and *CYP2C9* genotypes, which explained approximately 33.7% of overall warfarin dosage variability (Hillman et al., 2004). In 2005, they evaluated the feasibility of

applying the multivariate *CYP2C9* gene-based warfarin dosing model in clinical practice. Twenty patients received the standard initiation dose of 5mg/day. Eighteen patients were tested for *CYP2C9**1, *2 and *3 variants and received model-based initial dosing, determined by the multivariate model and any variant they may have (Hillman et al., 2005). They found that six adverse events occurred within the standard dose group and only two within the model-based group. Although their numbers were small they determined that the model-based dosing was feasible. In November of 2005, the Clinical Pharmacology Subcommittee, an FDA (Food and Drug Association) advisory committee, agreed that there is sufficient evidence to support the use of altered initiation doses of warfarin for patients with *CYP2C9* and *VKORC1* variants (Kimball Genetics Website). A label change for warfarin is currently underway to reflect this recommendation (Kimball Genetics Website).

In 2007 Kimball Genetics, Inc. launched a warfarin sensitivity DNA test. The test determines the presence of *CYP2C9*2* and *CYP2C9*3* and *VKORC1* (-1639 G>A) variants (Kimball Genetics Website). The aim of the test is to provide information about the genetic risk factors for over-anticoagulation and help achieve the correct maintenance dose faster (Kimball Genetics Website). Although helpful, this test focuses on three variants within the *CYP2C9* and *VKORC1* genes which are most common in Caucasian populations. Thus, limiting its informativity in other populations and excluding the influence of other genes and environmental factors on warfarin dosage.

A prospective study of up to 2000 patients is currently ongoing in the UK and aims at looking at all the genes involved in the mode of action of warfarin, assessing environmental factors including the clinical (age, gender, ethnicity, disease, concurrent

medication, adherence to treatment), pharmacological (*R*- and *S*-Warfarin levels), biochemical (vitamin K and epoxide levels) and haematological (clotting factor levels) phenotypes (Wadelius and Pirmohammed, 2007). In addition, this study aims at assessing the cost-effectiveness of pre-prescription genotyping, providing values for positive and negative prediction and numbers needed to screen (Wadelius and Pirmohammed, 2007). These results will provide the much-needed information to undertake prospective randomised controlled trials to assess the clinical utility of pre-prescription genotyping for warfarin (Wadelius and Pirmohammed, 2007).

1.6 Fetal Warfarin Syndrome (FWS)

Prosthetic heart valves cause hypercoagulable states, increasing the risk of thromboembolic complications (TEC) in patients with these heart prosthetic valves. Hypercoagulation is further increased in pregnancy. Warfarin effectively prevents these TEC but crosses the placenta during pregnancy, and is teratogenic, resulting in a specific constellation of malformations known as fetal warfarin syndrome (FWS) (Hall et al., 1980). The most constant malformations are nasal hypoplasia and stippled epiphyses due to the exposure of the fetus to warfarin within the first trimester (Hall et al., 1980). Other abnormalities involve the central nervous system (CNS) and eye, most likely as a result of warfarin taken during the second and third trimesters (Hall et al., 1980). In South Africa (SA), rheumatic fever is still common, which results in heart valve damage and eventual heart valve replacements in young women (Reviewed in: Gregersen, 2005). These young women require warfarin treatment, which then puts them at risk of having a pregnancy on warfarin.

A greater unbound fraction of warfarin has been found in the serum of pregnant women than non-pregnant women. It is this unbound fraction that crosses the placenta and causes teratogenic effects (Bajoria et al., 1996). Foetuses have high concentrations of bilirubin which displaces the unbound fraction of warfarin from albumin into serum. In addition they do not have the ability to produce water soluble warfarin metabolites for renal elimination, as their hepatic glucuronide pathway is immature (Bajoria et al., 1996). The exact pathogenesis of FWS, however, is unclear.

1.6.1 Incidence

Hall et al., (1980) estimated that, at best, 2/3 of babies born to mothers taking warfarin would be normal, 1/6 aborted or stillborn and a further 1/6 abnormal. A study carried out in SA in 1989, at Baragwanath Hospital in Johannesburg, determined that out of 50 pregnancies in 49 patients (all of whom received warfarin in the 1st and 2nd trimesters), 40% resulted in an abnormal pregnancy outcome and 4% of newborns were confirmed to have FWS (Sareli et al., 2000). No maternal deaths or TEC were associated with pregnancy in these patients (Sareli et al., 2000). In 2001, 49 patients were followed in the Western Cape of SA, 68% (24/49) received warfarin in the 1st trimester. 28% of these patients experienced pregnancy loss, while 6% of the live-borns were noted to have features of FWS (Hall et al., 2001). Three of these mothers died in the post-partum period, one due to accidental head injury and intracranial bleeding while on heparin and the other two due to TEC (Hall et al., 2001).

1.6.2 Alternative Anticoagulant and Current Regimens

An alternative to warfarin is unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). The administration of heparin is inconvenient, as patients have to be hospitalised (heparin is given intravenously), its administration is painful, expensive and is associated with a risk of bleeding, osteoporosis and heparin-induced thrombocytopaenia (HIT) (Ginsberg et al., 2001). During pregnancy heparin does not cross the placenta and has shown no relationship with FWS. When observing patients who had been switched from warfarin to heparin before or at six weeks of pregnancy no babies (0/108) had FWS. However, 4/36 babies, whose mothers were switched from warfarin to heparin after six weeks, had FWS (Ginsberg et al., 2001). These findings illustrate the importance of switching patients from warfarin to heparin, before six weeks of pregnancy, to prevent FWS. However, the administration of heparin during the first trimester in patients with mechanical heart valve prostheses shows high rates of maternal complications such as embolism and prosthetic valve thrombosis (Cotrufo et al., 2002).

Three anticoagulation regimens are currently available internationally to all pregnant women with prosthetic heart valves; these are:

1. Heparin used throughout pregnancy (and possibly before conception)
2. Warfarin used throughout pregnancy, changing to heparin at approximately 38 weeks gestation with a planned induction of labour
3. Heparin used during the 1st trimester (particularly between 6-9 weeks), switching back to warfarin from the 2nd trimester to 37 completed weeks of pregnancy, and then back to heparin until after planned delivery

Regimen 3, although the most recommended, is problematic as these patients usually require hospital admission for the duration of heparin administration as they require frequent monitoring, usually present after six weeks of gestation and warfarin exposure during the 2nd and 3rd trimesters may still result in abnormalities (Chan et al., 2000, Sadler et al., 2000, Ginsberg et al., 2001).

1.7 Aim and Impact of this study

Warfarin has been used for over 50 years and is most likely to remain the gold standard for the treatment of TEC for another decade or more. It is in the best interests of those who require such treatment to provide the best possible management of warfarin. Pre-administration pharmacogenetic testing of *CYP2C9* and *VKORC1* variants that alter warfarin dose in patients requiring warfarin has reduced the amount of adverse events in these patients and contributed to the determination of maintenance doses more efficiently (Kimball Genetics Website). These tests are based on variants that are present in a certain population at a high frequency and whose influence on warfarin dosage in known. No studies have yet determined the frequencies of these variants within the South African (SA) populations. Genetic variation is common amongst different populations and is usually higher in populations of sub-Saharan African origin than any other geographic region (Releford, 2001). Thus pharmacogenetic tests may need to be designed specifically for each population.

In 2005, Dr Nerine Gregersen of the Division of Human Genetics, School of Pathology, and Faculty of Health Science at the University of the Witwatersrand, submitted a research report for the degree of MSc (Med) in Genetic counselling entitled: The implications to women of childbearing age taking warfarin anticoagulation. Her project aimed at

determining the pregnancy outcomes in a cohort of 124 black urban South African women of childbearing age (followed at the Obstetric Cardiac Clinic at the Chris Hani-Baragwanath Hospital in Johannesburg), their awareness of the effects of warfarin in pregnancy, what management practices, as reported by them, had occurred with regard to their anticoagulation in pregnancy and what genetic counselling they had received (Gregersen, 2005). This project showed that 55.2% (123/223) of warfarin-exposed pregnancies resulted in the birth of an abnormal baby, spontaneous abortion or intrauterine death, estimating a FWS rate of 4.5-5.4% (Gregersen, 2005). Of these warfarin exposed pregnancies, 95% were reportedly exposed during the critical 6-10 week period of pregnancy, and less than 50% after 36 weeks (Gregersen, 2005).

Based on the findings of this project and the fact that *CYP2C9* and *VKORC1* variants are reported to account for a large proportion of warfarin dosage sensitivity, I aimed to determine the frequencies of all described variants within these two genes in the SA black population. Populations of African origin tend to have higher genetic variation than most other populations and thus I expected to find novel variants within these two genes. I then aimed to correlate these variants to dosage sensitivity and pregnancy outcomes in patients who had taken warfarin during pregnancy.

My specific aims were as follows:

1. Sequence all nine exons and intron/exon boundaries for *CYP2C9* and all three exons and intron/exon boundaries for *VKORC1* in South African black control and patient samples
2. Identify and determine the frequencies of known and novel *CYP2C9* and *VKORC1* variants

3. Compare the frequencies of these known and novel *CYP2C9* and *VKORC1* variants between the patient and control samples and to those in previous studies
4. Correlate these *CYP2C9* and *VKORC1* variants to warfarin dosage variability in the patients
5. Correlate these *CYP2C9* and *VKORC1* variants to pregnancy outcome in patients who had taken warfarin during pregnancy

Identifying known and novel *CYP2C9* and *VKORC1* variants in the South African black population will shed light on which variants, if any, are common in this population. Correlating these variants to warfarin dosage sensitivity and pregnancy outcomes will shed light on which variants influence warfarin dosage and pregnancy outcome in the South African black population. These results could eventually be used to design a pharmacogenetic test, specific to South African black patients, to identify patients with variants that alter warfarin dose and those with an increased risk of having a poor pregnancy outcome when taking warfarin. From this test, adjustments to warfarin dosage, specific to the patient, could be made to reduce adverse effects and determine the appropriate maintenance dose more efficiently. Similarly, appropriate counselling could be given to patients with increased risks of having a poor pregnancy outcome on warfarin.

2 SUBJECTS, MATERIALS AND METHODS

This chapter aims to describe all the subjects, methods and materials that were used in this project. Section 2.1 describes all the subjects that were used. Sections 2.2.1 – 2.2.5 describes the methods that were used to obtain sequences for all nine exons and intron/exon boundaries for the *CYP2C9* gene and all three exons and intron/exon boundaries for the *VKORC1* gene for all the patient and control samples. Section 2.2.6 describes how the sequences were analysed for new and previously described *CYP2C9* and *VKORC1* variants. Section 2.2.7 describes the methods and statistical models that were used to analyse the new and previously described *CYP2C9* and *VKORC1* variants and to correlate these variants to warfarin dosage and pregnancy outcomes.

2.1 Subjects

Two sample groups were used in this project, described in sections 2.1.1 and 2.1.2.

2.1.1 Patients

One hundred and thirteen blood samples were collected from black patients followed at the Obstetric Cardiac Clinic at the Chris Hani-Baragwanath Hospital in Johannesburg. These patients were part of a study carried out by Dr Nerine Gregersen, entitled: The implications to women of childbearing age taking warfarin anticoagulation. This project was submitted to the Faculty of Health Science at the University of the Witwatersrand as an MSc (Med) in Genetic Counselling, in 2005. Most of these patients were on warfarin as a result of artificial heart valves, six due to mitral valve repair, one due to mitral stenosis, one due to

hypertension and one post surgery for artificial valve canal defect and all had at least one pregnancy on warfarin. These patients were given new identity numbers: patients (P) 1 – 113, to maintain anonymity. Dr Gregersen obtained information on these patients' ages, their current warfarin dose (with the exception of P49, P51 and P111), a list of other drugs taken (described in section 3.2.1), pregnancy outcomes (with the exception of P51 and P111) and whether or not warfarin and/or heparin was taken during their pregnancies. Many of these patients had more than one pregnancy, shown in table 2.1.

Table 2.1: Number of pregnancies amongst the patients

Number of Pregnancies	Number of Patients (n = 111)
1	31 (28%)
2	30 (27%)
3	25 (23%)
4	10 (9%)
More than 4	15 (14%)

The total number of pregnancies, their outcomes and whether or not heparin and/or warfarin were taken during pregnancy is summarised in table 2.2. All ectopic pregnancies, termination of pregnancy (TOP) and pregnancies where only heparin was taken (highlighted in table 2.2) were excluded from our study.

Table 2.2: Summary of pregnancy information obtained for the patients (n=111)

Description	Normal Outcome	Poor Outcomes	Ectopic or TOP	Totals
Pregnancies on warfarin only	30 (25%)	83 (69%)	8 (6%)	121
Pregnancies on warfarin and heparin	45 (51%)	44 (49%)	0	89
Pregnancies on heparin only	2 (100%)	0	0	2
Pregnancies with neither warfarin nor heparin	67 (89%)	7 (9%)	1 (1%)	75
Total number of pregnancies	**144 (50%)**	**134 (47%)**	**9 (3%)**	**287**

Table 2.3 gives a summary of the number of patient samples and the analyses in which they were used in this study.

Table 2.3: Summary of the number of patient samples and the analyses in which they were used in this study

Analysis	Application	Number of Samples	Sections
Variant	Sequencing of all eight *CYP2C9* and three *VKORC1* intron/exon boundaries [1]	113	2.2.5
	Screening for new and previously described *CYP2C9* and *VKORC1* variants [1]	113	2.2.6, 3.1.1, 3.1.2
	HWE and Linkage Disequilibrium	113	2.2.7.1, 3.1.3, 3.1.4
	Comparison of patient and control samples	113	2.2.7.1, 3.1.5
Dosage	Correlating environmental factors to warfarin dosage	110 [2]	2.2.7.2, 3.2.1
	Correlating *CYP2C9* and *VKORC1* variants to warfarin dosage	110 [2]	2.2.7.2, 3.2.2
Pregnancy Outcomes	Correlating environmental factors to pregnancy outcome	108 [3]	2.2.7.3, 3.3.1
	Correlating *CYP2C9* and *VKORC1* variants to pregnancy outcome	108 [3]	2.2.7.3, 3.3.2

[1] 3 patient samples and 82 control samples could not be sequenced for *VKORC1* exon 2, described in section 2.2.5. [2] In the dosage analysis the sample number was 110 because I did not have dosage information for P49, P51 and P111. [3] In the pregnancy analysis the sample number was 108 because I did not have pregnancy information for P51 and P111 and excluded ectopic pregnancies and TOP.

2.1.2 Controls

One hundred random blood samples were obtained from the DNA bank in the Division of Human Genetics laboratory at the National Health Laboratory Service (NHLS) in Johannesburg. These samples represent the general South African black population (excluding Indian and mixed ancestry populations), with 50 of the samples being female and the other 50 being male. No distinction between the particular ethnic groups was made. Like the patient samples these samples were given new identity numbers: controls (C) 1-

100. Table 2.4 describes the number and the analyses in which the control samples were used in this project.

Table 2.4: Summary of the number of control samples and the analyses in which they were used in this study

Analysis	Application	Number of Samples	Sections
Variant	Sequencing of all eight *CYP2C9* and three *VKORC1* intron/exon boundaries [1]	100	2.2.5
	Screening for new and previously described *CYP2C9* and *VKORC1* variants [1]	100	2.2.6, 3.1.1, 3.1.2
	HWE and Linkage Disequilibrium	100	2.2.7.1, 3.1.3, 3.1.4
	Comparison of patient and control samples	100	2.2.7.1, 3.1.5

[1] 3 patient samples and 82 control samples could not be sequenced for *VKORC1* exon 2, described in section 2.2.5

2.2 Materials and Methods

All the solutions used in these methods are described in Appendix A.

2.2.1 DNA Extraction

All DNA samples were extracted based on a modified Salting-Out method (Miller et al., 1988) (protocol given in Appendix B). This technique uses various reagents, such as Sucrose-Triton X Lysing Buffer and Proteinase K, to lyse the membranes of DNA rich cells and remove contaminants such as proteins and cell debris. The protein is precipitated out of the solution by the addition of salt, while the DNA is precipitated from the solution by the addition of 100% ethanol and subsequently washed with 70% ethanol. The extracted DNA was re-suspended in TE buffer and concentrations determined using the Nano Drop

Spectrophotometer (ND - 1000). Working aliquots were diluted to 25ng/µl and kept in a 4˚C fridge while stock concentrations of each sample were kept in a -20˚C freezer.

2.2.2 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a technique that uses thermocycling to amplify a target region of DNA from small amounts of DNA via *in vitro* DNA replication. A basic reaction requires primer sequences that flank the region of interest, a thermostable DNA polymerase (*Taq* or Ampli *Taq* Gold (both supplied by Roche)), a PCR buffer specific to the polymerase used, dNTPs and DNA. The specific primers used for each PCR reaction varied for each amplicon and are described in section 2.2.2.1. In each reaction a blank sample (containing no DNA) was set up to ensure that the reagents were not contaminated or that no contamination had occurred during the setting up of the PCR.

2.2.2.1 Primer Sequences

The DNA sequences for the *CYP2C9* and *VKORC1* genes were obtained from the UCSC Genome Bioinformatics Database Website (NM_000771 and NM_024006 for *CYP2C9* and *VKORC1* respectively**).** The primers that were used for the *CYP2C9* gene were obtained from Blaisdell et al., 2004. However, due to the small size of exons 2 & 3 in this gene, I designed primers that flanked both exons in one amplicon, using Map Draw from the DNAStar software package (Supplied by Lasergene). The primers used for the *VKORC1* gene were obtained from D'Andrea et al., 2005.

All of the primers for each of the genes were "blasted" onto the human genome to ensure that they only bound to the region of interest. This was done using the BLAT and BLAST applications, from the UCSC Genome Bioinformatics Database Website and the National

Centre for Biotechnology Information (NCBI) Database. Any primers that bound to more than one region were modified (by either adding or removing base pairs) in Map Draw and re-blasted using BLAT and BLAST, until the primers only bound to the *CYP2C9* and *VKORC1* genes.

In Silico PCR from the UCSC Genome Bioinformatics Database Website was used to determine whether the primer sets amplified only the desired region of the human genome. All primers were obtained from Whitehead Scientific and Integrated DNA Technologies. The primer sets used for each amplicon are shown in table 5.1 in Appendix C.

2.2.2.2 PCR Conditions

The same PCR conditions were used for all amplicons, with the exception of their annealing temperatures. These temperatures were calculated from the primers' melting temperatures, provided by Whitehead Scientific and Integrated DNA Technologies, and the In Silico PCR programme on the UCSC Genome Bioinformatics Database Website. PCR reactions were carried out using which ever of the four thermocyclers (GeneAmp PCR system 9700, Eppendorf Mastercycler Gradient, AB 2720 Thermocycler or Eppendorf Thermocycler Gradient) were available in the Human Genetics laboratory. The reactions contained 1µl DNA (25ng), 1U Ampli *Taq* Gold DNA polymerase (Roche), 2.5mM Ampli *Taq* Gold polymerase buffer, 2.5mM $MgCl_2$ (Ampli *Taq*, buffer and $MgCl_2$ supplied by Roche), 0.125mM each dNTP (supplied by either Promega or Invitrogen), 10pM each primer and was made up to 25µl with ddH_2O. The dNTP mix, PCR mix and specific conditions for the different amplicons are all described in tables 5.2, 5.3 and 5.4, respectively, in Appendix C. Despite numerous trials I was unable to amplify exon 2 of the *VKORC1* gene for three of the patient samples and 82 of the control samples, possibly as a

result of DNA degradation. As no variation was seen in the 110 patient samples this was not pursued.

2.2.3 Detection on Agarose Gels

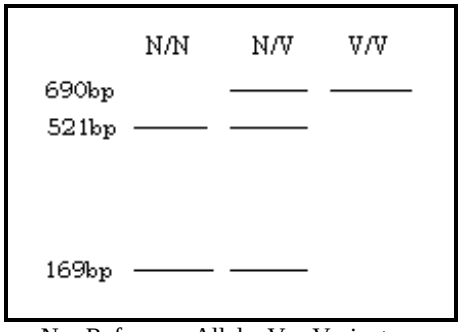
Amplification and the band sizes of all the PCR reactions were confirmed by 3% agarose gel electrophoresis (Appendix A). I mixed 5µl of each PCR product with approximately 2µl of Ficoll dye (Appendix A) before loading into the wells of agarose gels. Ficoll dye contains sucrose and Ficoll, making the solution denser so that it sinks to the bottom of the gel instead of floating out into the buffer. The Bromophenol blue dye enables one to visualise the sample while loading into the gel, and the migration of the dye front through the gel.

2.2.4 Restriction Digests

Some of the described variants in the *CYP2C9* gene are restriction fragment length polymorphisms (RFLP). RFLPs are variations or polymorphisms in specific regions of genomes that are detected by restriction enzymes. Restriction sites are DNA sequences that are recognised by restriction enzymes, responsible for cutting DNA either intrinsic or extrinsically. The presence of a particular allele of an RFLP, in a particular patient or control, is determined by digesting a particular PCR amplicon with the specific restriction enzyme (at the conditions of the enzyme specified by the supplier). The digested products are then viewed under UV light after running them on an agarose gel. Although RFLP assays are often cheaper and less time consuming than other SNP (single nucleotide polymorphism) detection assays, partial or incomplete digestion of the amplicons may

result in false positives or negatives. Thus it is important to run all digests with positive and negative controls.

Two RFLP assays were designed for the *CYP2C9*2* and *CYP2C9*5* variants. *CYP2C9*2* abolishes an *Ava*II restriction site within the *CYP2C9* exon 2&3 amplicon. Thus after digestion with *Ava*II (protocol given in table 5.5 in Appendix D) the three genotypes yield three different band patterns on a 3% agarose gel. The homozygous genotype (N/N) will yield two bands of 521bp and 169bp. The heterozygous genotype (N/V) will yield three bands, 690bp, 521bp and 169bp. The homozygous variant genotype (V/V) will yield only the 690bp band. Figure 2.1 represents a schematic diagram of a 3% agarose gel with the different genotypes for the *CYP2C9*2* variant after digestion with *Ava*II.

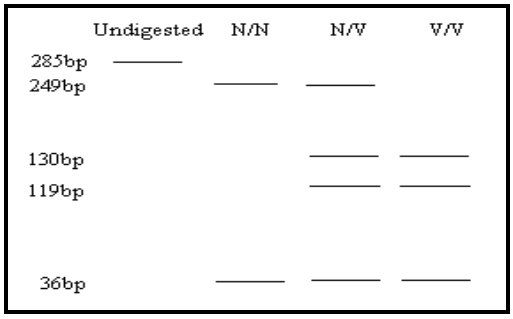


N = Reference Allele, V = Variant

Figure 2.1: Representation of a 3% agarose gel with the different genotypes for the *CYP2C9*2* variant after digestion with *Ava*II

Variant *CYP2C9*5* creates an additional *Alu*I restriction site within the *CYP2C9* Exon 7 amplicon. The undigested amplicon is 285bp in length. When digested with *Alu*I (protocol shown in table 5.6 in Appendix D) and run on a 3% agarose gel, the homozygous wild-type genotype (N/N) would have two bands of the sizes 249bp and 36bp. The heterozygous genotype (N/V) would have four bands of the sizes 249bp, 130bp, 119bp and 36bp. The

homozygous variant genotype (V/V) would show three bands of the sizes 130bp, 119bp and 36bp. Figure 2.2 represents a schematic diagram of a 3% agarose gel with the different genotypes for the *CYP2C9*5* variant after digestion with *Alu*I.



N = Reference Allele, V = Variant

Figure 2.2: Representation of a 3% agarose gel with the different genotypes for the *CYP2C9*5* variant after digestion with *Alu*II

The introduction of the capillary 3130xl genetic analyser (see section 2.2.5.4) into our department allowed us to identify known and new variants (undetected through the RFLP analysis) in the PCR amplicons in a much more efficient manner than RFLP assays. Thus all RFLP assays were stopped.

2.2.5 Sequencing

Sequencing is a method of determining the exact base composition of a particular strand of DNA. The most common method for DNA sequencing is an enzymatic method known as the Sanger method, named after its developer (Wilson and Walker, 2000). The modified Sanger method used, utilises fluorescently labelled dideoxynucleotide chain terminators (ddNTPs), which are similar to normal dNTPs but do not have a 3'-hydroxyl group. This missing 3'-hydroxyl group inhibits the formation of a phosphodiester bond at the 3' end,

preventing the extension of the DNA sequence during a sequencing specific PCR (Wilson and Walker, 2000). The fluorescently labelled ddNTPs (each with their own fluorochrome) enable one to have a single tube with all the ddNTPs and dNTPs, instead of four separate reactions like the original Sanger method. The incorporation of the ddNTP instead of a dNTP is a random event and results in a number of fragments of varying sizes, all having a common 5'-end (the primer). The cycle sequenced products are electrophoresed on a polyacrylamide gel or polymer matrix. The fluorescently labelled ddNTPs are detected by the laser on the Genetic Analyser (described in section 2.2.5.4) and translated into a G, T, A or C, depending on the wavelength detected. Sequences were obtained for all *CYP2C9* and *VKORC1* amplicons, for all the patient and control samples, with the exception of the three patient and 84 control samples for exon 2 of the *VKORC1* gene.

2.2.5.1 PCR Clean-up

Due to the sensitivity of the sequencing reaction, primers, excess reagents and non-specific products have to be removed from all PCR amplicons before sequencing can take place. A MultiScreen ® $PCR_{\mu 96}$ Cleanup Filter Plate from Millipore was used for all *CYP2C9* and *VKORC1* amplicons. The plate uses a size-exclusion membrane and vacuum filtration to remove unwanted particles from the PCRs. It requires no centrifugation or precipitation steps (Millipore, 2007). The protocol for this cleanup method is described in Appendix E. The cleaned samples were transferred to a new 96-well PCR plate and stored at 4˚C until needed.

2.2.5.2 Cycle Sequencing

Cycle sequencing was carried out, using BigDye Terminator Ready Reaction Mix (BDT) (Supplied by Roche), for all *CYP2C9* and *VKORC1* amplicons for both patients and

controls. All the cycle sequenced products were cleaned using a Montage SEQ_{96} Sequencing Reaction Clean up Kit (described in section 2.2.5.3), thus the cycle sequencing protocol (described table 5.7 in Appendix F) recommended by the Millipore/Montage clean up protocol was used to for all the cycle sequencing reactions. PCR bands that emitted high intensities on the agarose gels were diluted with an 1/8 reaction of BDT to a final volume of 10µl, while bands with lower intensity were diluted to a final volume of 5µl. The samples were then amplified using a cycle sequencing PCR protocol, which consists of 25 cycles of 96ºC for 30 seconds, 50ºC for 15 seconds and 60ºC for four minutes, and held at 10ºC.

2.2.5.3 Cycle Sequence Clean-up

All cycle sequenced products were cleaned using the Montage SEQ_{96} Sequencing Reaction Cleanup Kit from Millipore. This kit uses a patented size-exclusion membrane with vacuum filtration to remove unwanted molecules from 96 samples (larger plates are available), quicker than other protocols (Millipore, 2007). The protocol for this clean up procedure is described in Appendix G. Cleaned samples were transferred into a new 96-well plate and covered with an injection cover to be analysed on the Applied Biosystems 3130xl Genetic Analyser.

2.2.5.4 Genetic Analyser

Capillary sequencers, such as the Applied Biosystems 3130xl Genetic Analyser, use the same principles as a semi-automated sequencer in that they use a gel matrix to separate DNA fragments, which are then detected by a laser and the sequence compiled by a data processor (Imai et al., 1999). The difference between the 3130xl genetic analyser and a semi-automated genetic analyser is that the former uses a multi-capillary system rather

than a slab gel to separate the DNA fragments. This eliminates the polyacrylamide gel making step which is very time consuming and can be problematic (Imai et al., 1999). The multi-capillary system also allows one to run many more samples than one would be able to run on a slab gel system. The capillary genetic analyser that the Division of Human Genetics (NHLS and the University of the Witwatersrand) has, can take two 96 well plates and has 16 capillaries and therefore analyses 16 samples at a time.

2.2.5.4.1 Starting a Sequencing Run

Prior to initiating a sequencing run all cleaned cycle sequenced products were denatured at 95˚C for two minutes. This maintains the DNA strands in a single stranded form. Each of the troughs in the genetic analyser was checked for the correct volumes of either 1xTE buffer or ddH_2O. The 96 well plate(s) containing the cleaned cycle sequenced products were then placed in the Genetic Analyser in one of the two positions labelled A or B. The Foundation Data Collection Version 3.0 software package, (Applied Biosystems, Hitachi) provided with the 3130xl Genetic Analyser, was used to create and initiate each sequencing run. Appendix H gives a detailed description of how each sequencing run was created and started.

2.2.5.4.2 Retrieving a Sequencing Run

The Sequencing Analysis 5.2 software (Applied Biosystems, Hitachi) was used to analyse each sequence. This software, unlike the semi-automated model, automatically tracks, extracts and analyses each sequence. Appendix I gives a detailed description of how samples (run on the 3130xl genetic analyser) were retrieved and analysed.

2.2.6 Analysis of DNA Sequences

The sequences for all patient and control samples for each of the *CYP2C9* and *VKORC1* amplicons were compared to a reference sequence for that region, using the SeqMan programme from the DNAStar software package (Lasergene). All references sequences were obtained from UCSC Genome Bioinformatics Database Website (NM_000771 and NM_024006 for *CYP2C9* and *VKORC1* respectively**)**. The SeqMan programme aligns all sequences of the same size and base composition and highlights any changes in the sequences (such as base substitutions), as compared to the reference sequence. Any changes in the DNA sequences that were not previously described variants were labelled according to the position of the change and later characterised (described in section 2.2.6.1).

2.2.6.1 Novel Variants

The first step in characterising these novel variants was to determine whether they were in an intron or exon. This was determined using the sequences obtained from the UCSC Genome Bioinformatics Database Website, as they specify intron/exon boundaries. If the variants were found in an exon I determined whether or not they resulted in an amino acid change, using the MapDraw programme in the DNAStar software package (Lasergene). Those that were found in introns were placed into three different splice site predictor sites (Berkley Drosophila Genome Project (BDGP) Splice Site Predictor, Alex Dong Li's Splice Site Finder and NetGene2 Server). These sites use specific algorithms to predict intron splice sites within a specific region of DNA. Any variants found within these predicted splice sites are then likely to affect the splicing of that specific intron, by creating or removing a splice recognition site.

2.2.7 Statistics

This project had three main aims: 1) Determine the frequency of known and new *CYP2C9* and *VKORC1* variants, 2) Correlate these variants to weekly dosage in patients on warfarin and 3) Correlate these variants to the pregnancy outcome of patients taking warfarin during pregnancy. The analysis for each aim will be discussed separately in sections 2.2.7.1, 2.2.7.2 and 2.2.7.3, respectively. Following the generation, compilation and basic analysis of the data, statistical tests and models were generated during a two day consultation with Dr Van Der Merwe (from the Biostatics Department of the Medical Research Council of South Africa, Cape Town). All p-values (probability values) of 0.05 or below were taken as significant. Base R and R software packages version 0.2-3 (genetics, dgc_genetics, LDheatmap, haplo.stats and MASS) were used to carry out all statistical analyses (Venables and Ripley, 2002; Sinnwell and Schaid, 2005; Warnes and Leisch, 2005; Clayton., 2006).

2.2.7.1 Variant Analysis

Genotype and allele frequencies were calculated for the patients, controls and the total samples (both patients and controls together). Based on the type of analysis (correlating variants to dosage and pregnancy outcome) and the large number of variants I identified, I excluded all the variants that showed an allele frequency of less than 0.02 in the sample groups, from further analysis due to insufficient numbers. In addition variants that are present at a low frequency within a population may not be useful for pre-administration pharmacogenetic testing of that population; however, these variants may have significant effects in individuals with these variants.

An exact test (HWE.exact in R, genetics library) was used to check for Hardy-Weinberg equilibrium (HWE) for each of the variants in each of the sample groups (patients, controls and both patients and controls combined). HWE is based on the principle that relative proportions of different genotypes remain constant from one generation to the next. Heatmaps, which are graphs that show the probability of linkage disequilibrium between the different variants, were created for all the sample groups. Linkage disequilibrium refers to the occurrence in a population of certain combinations of linked alleles in greater proportion than expected from the allele frequencies at the loci (McGraw-Hill, 2002). Linkage disequilibrium was calculated using the LD function in R, genetics library (mentioned in section 2.2.7) and measured using D'. The LD function estimates the extent of linkage disequilibrium for a single pair of genotypes (R, genetics help file).

Two tests were used to determine whether or not there were any significant differences in the genotype and allele frequencies of the *CYP2C9* and *VKORC1* variants between the patient and control groups. The first test, Fisher's exact test, was used to test the differences between patients and controls based on their genotype frequencies. This test was the most suitable as some of the variants were observed at very low genotype frequencies in the control and patient samples. The second test, Cochran/Armitage trend test was used to test the differences between the patients and controls based on their allele frequencies. This test was used as it capable of detecting more trends than a normal chi-squared test.

2.2.7.2 Warfarin Dosage Variability Analysis

Warfarin maintenance dosage can be influenced by a number of environmental and genetic factors. The only environmental factors, on which information was obtained during Dr

Gregersen's study which may influence warfarin dosage, were age and concomitant medication (listed in section 3.2.1). Our aim in this analysis was to determine whether any of the known and new *CYP2C9* and *VKORC1* variants influence warfarin maintenance dosage in these patients. In order to accurately predict the influence of these variants on warfarin dosage, I needed also to determine the level of influence the patients' age and concomitant medications have on warfarin maintenance dosage in these patients.

2.2.7.2.1 Environmental Factors

Box plots were created to graphically depict the relationship between patients that were or were not taking a concomitant drug and warfarin dosage. The influence these concomitant drugs have on warfarin maintenance dosage was tested using a Wilcoxon test. This test is similar to a t-test and involves the comparison of differences in measures of two related samples. It was used to determine if there was any significant difference in warfarin maintenance dosage in the patients that were taking the particular drug of interest, to those that were not taking the drug. The influence the patients' age has on warfarin maintenance dosage was tested using the linear models (described in section 2.2.7.2.2).

2.2.7.2.2 CYP2C9 and VKORC2 Variants

Box plots were created to depict the relationship between the particular variants and warfarin dosage. The influence the particular variants have on warfarin dosage was tested using three different methods: a Kruskal-Wallis test, adapted linear models and haplo.stats analysis. The Kruskal-Wallis test is an extension of the Wilcoxon test but is capable of comparing more than two samples. Thus was used to compare the median maintenance dosage between the three genotypes of a particular variant.

The adapted linear models use logistic regression adjusting for the effects of age and concomitant medication on warfarin maintenance dosage. Logistic regression provides estimates of the size of genotype effects by treating the disease status as the outcome variable and the genotype as an explanatory variable (described by Clayton in the R package version 0.5). Using these models one is able to adjust for prognostic factors, which if ignored may lead to errors in estimating treatment differences (Hastie and Tibshirani, 2007).

A haplotype refers to a series of alleles found at linked loci on a single chromosome (Strachan and Read, 2004). Haplo.stats, run through the R software programme was used to carry out all the haplo.stats analysis used in this project. It is a suite of S-PLUS/R routines for the analysis of indirectly measured haplotypes/allele combinations (Sinnwell and Schaid, 2005). This statistical method assumes that all the subjects are unrelated, the haplotypes are ambiguous (due to unknown linkage phase of the genetic markers), and that the genetic markers are codominant (Sinnwell and Schaid, 2005). In this analysis the haplo.stats results were used to determine whether certain allele combinations alter warfarin maintenance dosage. The first analysis incorporated all 12 *CYP2C9* variants, followed by the analyses of only six variants at a time, i.e. variant 1 – 6, 2 – 7, 3 – 8 etc. Secondly I looked at both *VKORC1* variants and their effect on warfarin maintenance dosage. Lastly I looked at both the *CYP2C9* and *VKORC1* variants with respect to warfarin maintenance dosage.

2.2.7.3 Pregnancy Outcome Analysis

Most of the patients had more than one pregnancy (the frequencies of which can be seen in table 2.1 in section 2.1.1) with different outcomes. Bar graphs were created, using Excel (Microsoft, Windows) to show the frequency of poor (which includes miscarriages, stillbirths and abnormal live-borns) and normal pregnancy outcomes for the first three pregnancies (pregnancies above three were not analysed because of a small sample size). The aim of this analysis was to determine whether any of the known or new *CYP2C9* and *VKORC1* variants influence pregnancy outcome when on warfarin. It is known, however, that pregnancy outcome can be influenced by many environmental and genetic factors. The only environmental factors I was able to account for in this study (taken from Dr Gregersen's data) were the patients' age and whether they took heparin and/or warfarin during their pregnancy.

2.2.7.3.1 Environmental Factors

Box plots were created to graphically depict the effects of the patients' ages on pregnancy outcome. Bar graphs were created, using Excel (Microsoft, Windows), to show the relationship between warfarin maintenance dosage and pregnancy outcome. Generalised linear models (described in section 2.2.7.3.2) were used to determine the influence of warfarin and/or heparin on pregnancy outcome.

2.2.7.3.2 CYP2C9 and VKORC1 Variants

When determining the influence of the particular variants on pregnancy outcome I could only analyse one pregnancy outcome as no statistical software is yet available to analyse more than one outcome for a single patient. Thus the statistical analyses used to determine

the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome were based on the pregnancy outcomes after the 1st pregnancy. Box plots were created to show the relationship between the variants and poor and normal pregnancy outcomes. The influence of these variants on pregnancy outcome was tested using three types of analyses: generalised linear models, interaction models and haplo.stats analyses.

The generalised linear models determined the effects of the different *CYP2C9* and *VKORC1* variants on pregnancy outcome (independent of warfarin taken during the pregnancy), adjusting for the effect of the patients' age, number of pregnancies and whether or not heparin and/or warfarin was used during the pregnancy. Interaction models determine the effect of a particular variant on pregnancy outcome when warfarin is taken during the pregnancy, adjusting for the effect of the patients' age, number of pregnancies and whether or not heparin and/or warfarin was used during the pregnancy.

Haplo.stats analyses were carried out for both the *CYP2C9* and *VKORC1* variants separately, using four *CYP2C9* variants at a time or the two *VKORC1* variants. I compared the allele combination frequency of the patients that had poor pregnancy outcomes to those who had normal pregnancy outcomes when warfarin was and was not taken during pregnancy.

3 RESULTS

This chapter aims to describe all the results obtained from the control and patient samples using the methods described in section 2. Section 3.1 describes all the known and new variants I observed in the sequences obtained for all the *CYP2C9* and *VKORC1* amplicons. Section 3.2 describes the correlation of these variants to warfarin maintenance dosage. Section 3.3 describes the correlation of these variants to pregnancy outcomes in patients taking warfarin during pregnancy. In all of the statistical analyses p-values of below 0.05 were considered significant and significant comparisons are highlighted in all the tables. The P-values for all the tests and linear models for each analysis (variant, warfarin dosage variability and pregnancy outcomes (described in sections in section 3.1 – 3.3) are shown in appendices J – L, respectively. The raw data collected during this project and for the use of this project may be seen in Appendix M. Pictures of some agarose gels and electropherograms produced during the project may be seen in Appendix N.

3.1 Variant Analysis

The aim of this analysis was to identify novel and previously described *CYP2C9* and *VKORC1* variants, and to determine their frequencies in the patient and control samples; and to compare these frequencies between the two sample groups. Section 3.1.1 describes the *CYP2C9* variants observed in the sample groups. Section 3.1.2 describes the *VKORC1* variants observed in the sample groups. All the variants that were observed at a frequency of ≥ 0.02 were used for further analysis and are described in sections 3.1.3-3.1.5, 3.2 and 3.3.

3.1.1 *CYP2C9* Variants

3.1.1.1 Previously Described Variants

New *CYP2C9* variants are being described on a continuous basis. To date there are 30 described variants (listed in table 1.2, section 1.3). Of the 30 known variants I observed six in the control samples and four in the patient samples. Their frequencies are shown in table 3.1.

Table 3.1: Genotype and allele frequencies of the previously described *CYP2C9* variants observed in the patient and control samples

Variant	Patient Samples (n =113)				Control Samples (n = 100)			
	Genotype		Allele		Genotype		Allele	
	N^1/V^2	V^2/V^2	N^1	V^2	N^1/V^2	V^2/V^2	N^1	V^2
*CYP2C9*3* (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(1) 0.01	(0) 0	(199) 0.99	(1) 0.01
*CYP2C9*5* (n) frequency	(0) 0	(0) 0	(226) 1	(0) 0	(2) 0.02	(0) 0	(198) 0.99	(2) 0.01
*CYP2C9*6* (n) frequency	(0) 0	(0) 0	(226) 1	(0) 0	(1) 0.01	(0) 0	(199) 0.99	(1) 0.01
*CYP2C9*8* (n) frequency	(26) 0.23	(2) 0.02	(196) 0.87	(30) 0.13	(16) 0.16	(0) 0	(184) 0.92	(16) 0.08
*CYP2C9*9* (n) frequency	(36) 0.32	(2) 0.02	(186) 0.82	(40) 0.18	(18) 0.18	(3) 0.03	(176) 0.88	(24) 0.12
*CYP2C9*11* (n) frequency	(8) 0.07	(0) 0	(218) 0.96	(8) 0.04	(7) 0.07	(1) 0.01	(191) 0.96	(9) 0.04

[1]N = Normal, [2]V = Variant
Highlighted variants = observed at an allele frequency of ≥ 0.02, thus used for further analysis

In table 3.1, although variant *CYP2C9*5* was observed in the control samples at a frequency of 0.02 it was excluded from further analysis because it was not observed in the patient sample group.

3.1.1.2 Novel Variants

I observed 27 novel, previously unidentified, *CYP2C9* variants (24 in the patient samples and 19 in the control samples). Eight of these were observed within exons 1, 2, 3, 7 and 9, and are described in section 3.1.1.2.1. The remaining 19 were observed in the intronic regions of amplicons 1, 2&3, 4, 6, 8 and 9, and are described in section 3.1.1.2.2.

3.1.1.2.1 Coding Sequence Variants

The eight novel variants that were observed within the *CYP2C9* exons were analysed to determine whether or not they altered an amino acid. Four of these variants are silent mutations (do not change the amino acid). The other four are missense mutations (change the amino acid). Table 3.2 describes each of these variants.

Table 3.2: Description of all the novel *CYP2C9* silent and missense mutations observed within the patient and control samples

Exon	SNP [1]	Amino Acid Change
1	12803 A>G	I42V
2	15906 A>G	I74V
2	15913 T>A	V76Q
3	16247 G>T	T130T
7	55198 T>C	I327T
9	62875 C>T	A441A
9	62941 C>T	D463D
9	62977 A>T	G465G

The four missense mutations are highlighted in the table

[1] SNP = single nucleotide polymorphism/change in the DNA strand

Figure 3.1 illustrates the Isoleucine to Valine amino acid change brought about by the I42V and I74V missense mutations.

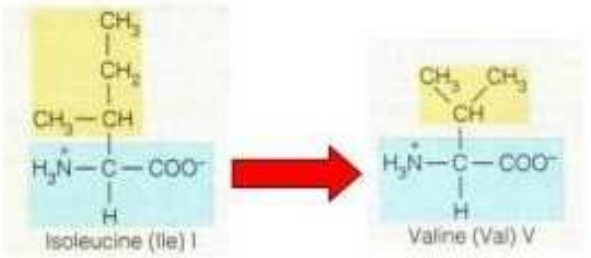


Figure 3.1: Isoleucine changed to a Valine (I42V, I74V), (Mathews et al., 2000)

Isoleucine and Valine both have non-polar side chains, shown in yellow in figure 3.1. Isoleucine, however, has an additional CH_2 as compared to Valine.

Figure 3.2 illustrates the Valine to Glutamine mutation at amino acid 76.

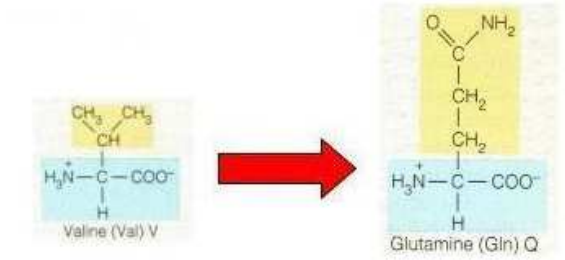


Figure 3.2: Valine changed to a Glutamine (V76Q), (Mathews et al., 2000)

The Valine to Glutamine mutation results in the substitution of a non-polar amino acid to polar amino acid. Similarly, the I327T mutation results in the substitution of a non-polar amino acid to a polar amino acid, shown in figure 3.3.

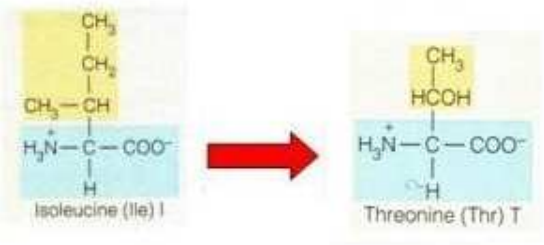


Figure 3.3: Isoleucine change to Threonine (I327T), (Mathews et al., 2000)

Table 3.3 shows the genotype and allele frequencies of the four novel silent mutations and four novel missense mutations in the patient and control samples.

Table 3.3: Genotype and Allele frequencies of the four novel silent and four novel missense mutations observed in the patient and control samples

Variant	Patient Samples (n =113)				Control Samples (n = 100)			
	Genotype		Allele		Genotype		Allele	
	N^1/V^2	V^2/V^2	N^1	V^2	N^1/V^2	V^2/V^2	N^1	V^2
I42V (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(0) 0	(0) 0	(200) 1	(0) 0
I74V (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(0) 0	(0) 0	(200) 1	(0) 0
V74Q (n) frequency	(0) 0	(0) 0	(226) 1	(0) 0	(3) 0.03	(0) 0	(197) 0.985	(3) 0.015
T130T (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(2) 0.02	(0) 0	(198) 0.99	(2) 0.01
I327T (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(0) 0	(0) 0	(200) 1	(0) 0
A441A (n) frequency	(15) 0.13	(0) 0	(211) 0.93	(15) 0.07	(15) 0.15	(0) 0	(185) 0.93	(15) 0.07
D463D (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(1) 0.01	(0) 0	(199) 0.99	(1) 0.01
G465G (n) frequency	(3) 0.03	(0) 0	(223) 0.99	(3) 0.01	(2) 0.02	(0) 0	(198) 0.99	(2) 0.01

[1]N = Normal, [2]V = Variant
Highlighted variant = observed at an allele frequency of ≥ 0.02, thus used for further analysis

3.1.1.2.2 Non-Coding Sequence Variants

The remaining 19 novel non-coding sequence variants were run through three splice site predictor sites (described in section 2.2.6.1). The scores or confidence percentages for each of the splice site predictors, is a measure of the likelihood of a splice site in a particular region of DNA. Thus, splice sites that have a high score or confidence percentage are more

likely to be actual splice sites than those with lower scores. The cut off values for each of the predictor sites are as follows: Fruitfly – 40, Sickkids – 65 and NetGene2 – 50. Nine of the 19 remaining variants are found within possible splice sites and therefore may affect the splicing of that region. Table 3.4 shows the results of the searches using the three splice site predictor sites for the nine variants.

Table 3.4: Summary of the Splice Site predictor website searches for the nine possible splice site mutations

Variant	Fruit Fly*		Genet Sickkids†		Net Gene2‡	
	Score	Type	Score	Type	Confidence	Type
12930 T>C	95	Donor	74.1	Donor	50-95%	Donor
16090 T>C	None	None	66.1	Acceptor	50-95%	Donor (- strand)
16094 C>A	None	None	66.1	Acceptor	50-95%	Donor (- strand)
21711 G>C	None	None	68.9	Acceptor	50-95%	Donor (- strand)
46028 A>G	None	None	69.5	Donor	None	None
46092 C>T	None	None	73.2	Acceptor	None	None
60175 A>G	None	None	79.9	Donor	50-95%	Donor
63143 C>G	None	None	79.5	Acceptor	None	None
63180 C>T	None	None	74.3	Acceptor	None	None

References: *BDGP Splice Site Predictor, †Alex Dong Li's Splice Site Finder, ‡NetGene2 Server
Cut off values for each site: Fruitfly = 40, Sickkids = 65, NetGene2 = 50

Table 3.5 shows the genotype and allele frequencies of these nine possible splice site mutations in the patient and control samples.

Table 3.5: Genotype and Allele frequencies of the nine novel possible splice site mutations observed in the patient and control samples

Variant	Patient Samples (n =113)				Control Samples (n = 100)			
	Genotype		Allele		Genotype		Allele	
	N^1/V^2	V^2/V^2	N^1	V^2	N^1/V^2	V^2/V^2	N^1	V^2
12930 T>C (n) frequency	(25) 0.22	(1) 0.01	(199) 0.88	(27) 0.12	(19) 0.19	(3) 0.03	(175) 0.88	(25) 0.12
16090 T>C (n) frequency	(27) 0.24	(2) 0.02	(195) 0.86	(31) 0.14	(24) 0.24	(3) 0.03	(170) 0.85	(30) 0.15
16094 C>A (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(2) 0.02	(0) 0	(198) 0.99	(2) 0.01
21711 G>C (n) frequency	(22) 0.19	(3) 0.03	(198) 0.88	(28) 0.12	(19) 0.19	(2) 0.02	(177) 0.89	(23) 0.11
46028 A>G (n) frequency	(39) 0.35	(13) 0.11	(161) 0.71	(65) 0.29	(40) 0.40	(3) 0.03	(154) 0.77	(46) 0.23
46092 C>T (n) frequency	(4) 0.04	(0) 0	(222) 0.98	(4) 0.02	(4) 0.04	(0) 0	(196) 0.98	(4) 0.02
60175 A>G (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(0) 0	(0) 0	(200) 1	(0) 0
63143 C>G (n) frequency	(0) 0	(0) 0	(226) 1	(0) 0	(1) 0.01	(0) 0	(199) 0.99	(1) 0.01
63180 C>T (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(0) 0	(0) 0	(200) 1	(0) 0

[1]N = Normal, [2]V = Variant

Highlighted variants = observed at an allele frequency of ≥ 0.02, thus used for further analysis

The effects of the ten remaining variants are yet to be determined. Table 3.6 shows the genotype and allele frequencies of these ten variants.

Table 3.6: Genotype and Allele frequencies of the ten novel variants, whose effect on Cytochrome P450 is unknown, observed in the patient and control samples

Variant	Patient Samples (n =113)				Control Samples (n = 100)			
	Genotype		Allele		Genotype		Allele	
	N^1/V^2	V^2/V^2	N^1	V^2	N^1/V^2	V^2/V^2	N^1	V^2
16060 T>C (n) frequency	(0) 0	(0) 0	(226) 1	(0) 0	(1) 0.01	(0) 0	(199) 0.99	(1) 0.01
16179 T>A (n) frequency	(22) 0.19	(3) 0.03	(198) 0.88	(28) 0.12	(27) 0.27	(1) 0.01	(171) 0.86	(29) 0.14
21748 G>A (n) frequency	(2) 0.02	(0) 0	(224) 0.99	(2) 0.01	(1) 0.01	(0) 0	(199) 0.99	(1) 0.01
60225 T>A (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(0) 0	(0) 0	(200) 1	(0) 0
60272 T>C (n) frequency	(14) 0.12	(0) 0	(212) 0.94	(14) 0.06	(14) 0.14	(0) 0	(186) 0.93	(14) 0.07
60318 C>T (n) frequency	(3) 0.03	(0) 0	(223) 0.99	(3) 0.01	(2) 0.02	(0) 0	(198) 0.99	(2) 0.01
60328 A>G (n) frequency	(2) 0.02	(0) 0	(224) 0.99	(2) 0.01	(1) 0.01	(0) 0	(199) 0.99	(1) 0.01
63092 C>T (n) frequency	(1) 0.01	(0) 0	(225) 0.99	(1) 0.01	(0) 0	(0) 0	(200) 1	(0) 0
63113 C>T (n) frequency	(22) 0.19	(3) 0.03	(198) 0.88	(28) 0.12	(17) 0.17	(0) 0	(183) 0.92	(17) 0.08
63169 G>A (n) frequency	(2) 0.02	(0) 0	(224) 0.99	(2) 0.01	(0) 0	(0) 0	(200) 1	(0) 0

[1]N = Normal, [2]V = Variant

Highlighted variants = observed at an allele frequency of ≥ 0.02, thus used for further analysis

3.1.2 *VKORC1* Variants

3.1.2.1 Previously Described Variants

VKORC1 is a relatively newly identified gene. To date there are 13 described variants within this gene (described in table 1.3, section 1.4). The -1639 G>A promoter and Intron one 1173 C>T variants were not screened because they were described after this study had

been designed and they are found outside of our primer sequences. Of the 11 remaining variants I only observed three within the patient and control sample groups. Their frequencies are shown in table 3.7.

Table 3.7: Genotype and Allele frequencies of the previously described *VKORC1* variants observed in the patient and control samples

Variant	Patient Samples					Control Samples				
	(n)	Genotype		Allele		(n)	Genotype		Allele	
		N^1/V^2	V^2/V^2	N^1	V^2		N^1/V^2	V^2/V^2	N^1	V^2
V66M (n) frequency	**110**	(1) 0.01	(0) 0	(219) 0.99	(1) 0.01	**18**	(1) 0.01	(0) 0	(35) 0.97	(1) 0.03
L120L (n) frequency	**113**	(42) 0.37	(8) 0.07	(168) 0.74	(58) 0.26	**99**	(33) 0.33	(3) 0.03	(159) 0.80	(39) 0.20
3730 G>A (n) frequency	**113**	(53) 0.47	(23) 0.20	(127) 0.56	(99) 0.44	**100**	(43) 0.43	(21) 0.21	(113) 0.57	(85) 0.43

[1]N = Normal, [2]V = Variant. Highlighted variants = observed at an allele frequency of ≥ 0.02, thus used for further analysis. The sample sizes (shown in bold (n)) are different because some of the samples failed to amplify

No novel variants were found within this gene.

All of the *CYP2C9* and *VKORC1* variants (highlighted in tables 3.1, 3.3, 3.5, 3.6 and 3.7, in sections 3.1.1 and 3.1.2) observed at an allele frequency of ≥ 0.02 were used for further analysis. They were given new identity numbers (1-14) (listed in table 3.8) to make the representation of results clearer.

Table 3.8: List of the previously described and novel *CYP2C9* and *VKORC1* variants that were used for comparison and correlation analyses

New Identity Number	Described/Novel	Gene	Variant	Type of Mutation
1	Described	*CYP2C9*	*CYP2C9*8*	R150H
2	Described	*CYP2C9*	*CYP2C9*9*	H251R
3	Described	*CYP2C9*	*CYP2C9*11*	R355W
4	Novel	*CYP2C9*	12930 T>C	Possible Splice Site
5	Novel	*CYP2C9*	16090 T>C	Possible Splice Site
6	Novel	*CYP2C9*	16179 T>A	Unknown
7	Novel	*CYP2C9*	21711 G>C	Possible Splice Site
8	Novel	*CYP2C9*	46028 A>G	Possible Splice Site
9	Novel	*CYP2C9*	46092 C>T	Possible Splice Site
10	Novel	*CYP2C9*	60272 T>C	Unknown
11	Novel	*CYP2C9*	62875 C>T	A441A
12	Novel	*CYP2C9*	63113 C>T	Unknown
13	Described	*VKORC1*	8773 C>T	L120L
14	Described	*VKORC1*	9041 G>A	3730 G>A (3'UTR)

3.1.3 Comparison of Genotype and Allele frequencies of the 14 *CYP2C9* and *VKORC1* variants in the patient and control samples

Fisher's exact and Cochran/Armitage tests (described in section 2.2.7.1) were used to test whether there were any significant differences between genotype and allele frequencies of the 14 variants in the patient and control sample groups, respectively. No significant differences were found between the two groups, confirming that they are from the same South African Black population. Thus any findings in the dosage and pregnancy outcome analyses (in which only the patient samples were used, see table 2.3 in section 2.1.1) should apply to the general South African Black population. The p-values from all these tests may be seen in table 5.12 in Appendix J.

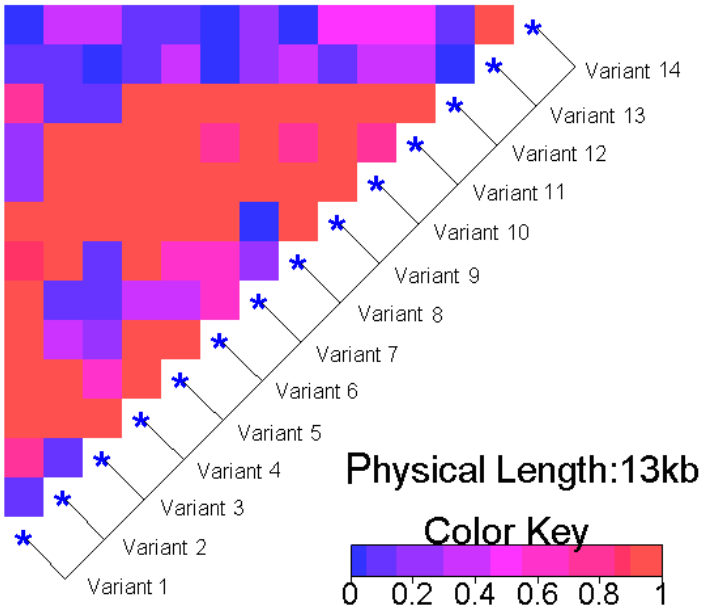
3.1.4 Hardy-Weinberg Equilibrium (HWE)

An exact test (mentioned in section 2.2.7.1) was used to test for compliance with Hardy-Weinberg expectation at each of the 14 loci described in table 3.8, in the patients, controls

and both patients and controls combined. From these results I determine that the genotype proportions at each of the 14 loci do not differ significantly from those predicted by the Hardy-Weinberg law (from the observed allele frequencies). This indicates that these variants are in HWE. The p-values for each of the sample groups can be seen in table 5.8 in Appendix J.

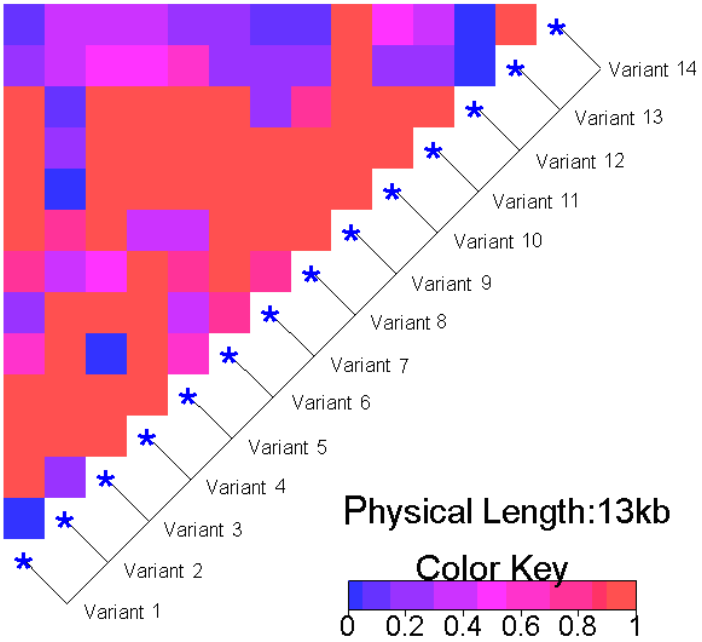
3.1.5 Linkage Disequilibrium (LD)

Linkage disequilibrium (described in section 2.2.7.1) was tested for between pairs of alleles at all 14 loci in all the sample groups. Figures 3.4, 3.5 and 3.6 show the heatmaps (described in section 2.2.7.1) created for all the sample groups. The p-values for these groups may be seen in tables 5.9 - 5.11 in Appendix J. In each table and figure a value of 1 shows LD, 0 shows no LD.



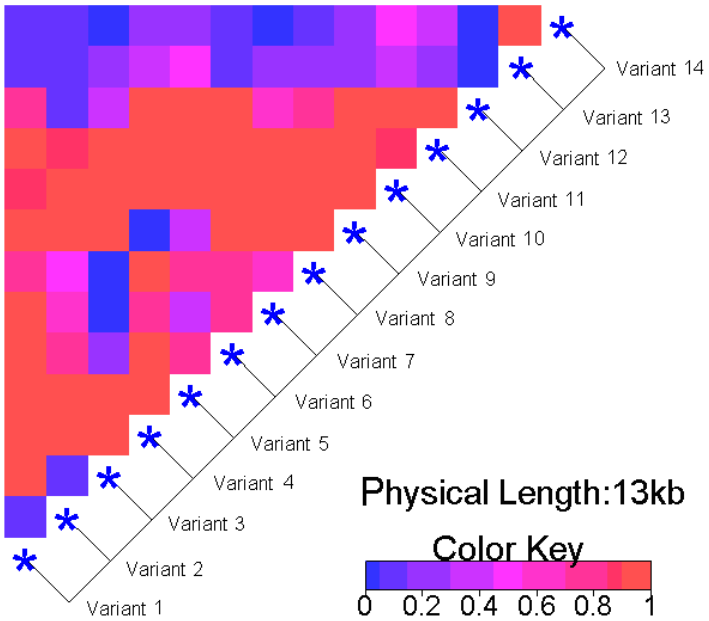
1 = LD; 0 = no LD

Figure 3.4: Linkage Disequilibrium Heatmap for the control samples



1 = LD; 0 = no LD

Figure 3.5: Linkage Disequilibrium Heatmap for the patient samples



1 = LD; 0 = no LD

Figure 3.6: Linkage Disequilibrium Heatmap for both the patient and control samples

The colour key in each of the heatmaps represents the p-values of the LD. Variants 13 & 14 are found on a different gene on a different chromosome from variants 1-12. Both of these genes are relatively small (three and nine exons, respectively). Thus I expected to see variants 13 & 14 in LD with each other; and similarly variants 1-12 in LD with each other. The LD between the different variants for each gene was analysed using the LD function in R at the same time, for convenience. However, we are not measuring the LD between the genes (as they are on different chromosomes) but rather the LD of the different variants within each of the genes.

3.2 Warfarin Dosage Variability Analysis

The aim of this analysis is to determine whether any of the 14 *CYP2C9* and *VKORC1* variants (described in table 3.8 in section 3.1.2.1) influence warfarin dosage sensitivity in the patient samples (n = 110). Figure 3.7, illustrates the distribution of warfarin dosage, in mg/week, amongst the patients. Dosage was measured in mg/week instead of mg/day as most of the patients alternate their daily dosage.

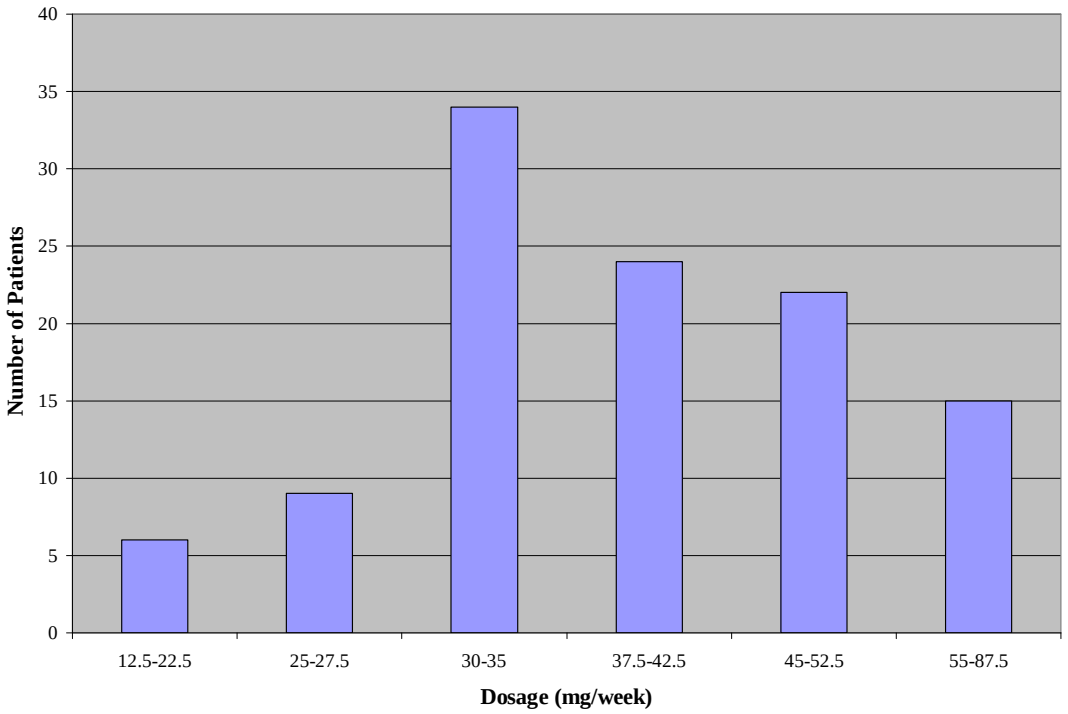


Figure 3.7: Distribution of warfarin dosage (in mg/week) in the patients (n = 110)

Based on the literature and our data I divided the patients into three dosage groups: low, average and high. Table 3.9 describes these groups and the number of patients in each group.

Table 3.9: Description of the three dosage groups and the number of patients in each group

Group	Dosage (mg/week)	Number of Patients (n = 110)
Low	12.5 – 27.5	(15) 14%
Average	30 – 42.5	(58) 53%
High	45 – 87.5	(37) 34%

This variability in warfarin dosage may be explained by a number of environmental and genetic factors. The two environmental factors, that I could account for in this project, were age and concomitant medication, discussed in section 3.2.1.

3.2.1 Environmental Factors

3.2.1.1 Age

Figure 3.8 shows the distribution of ages amongst the patients currently (the median being 40).

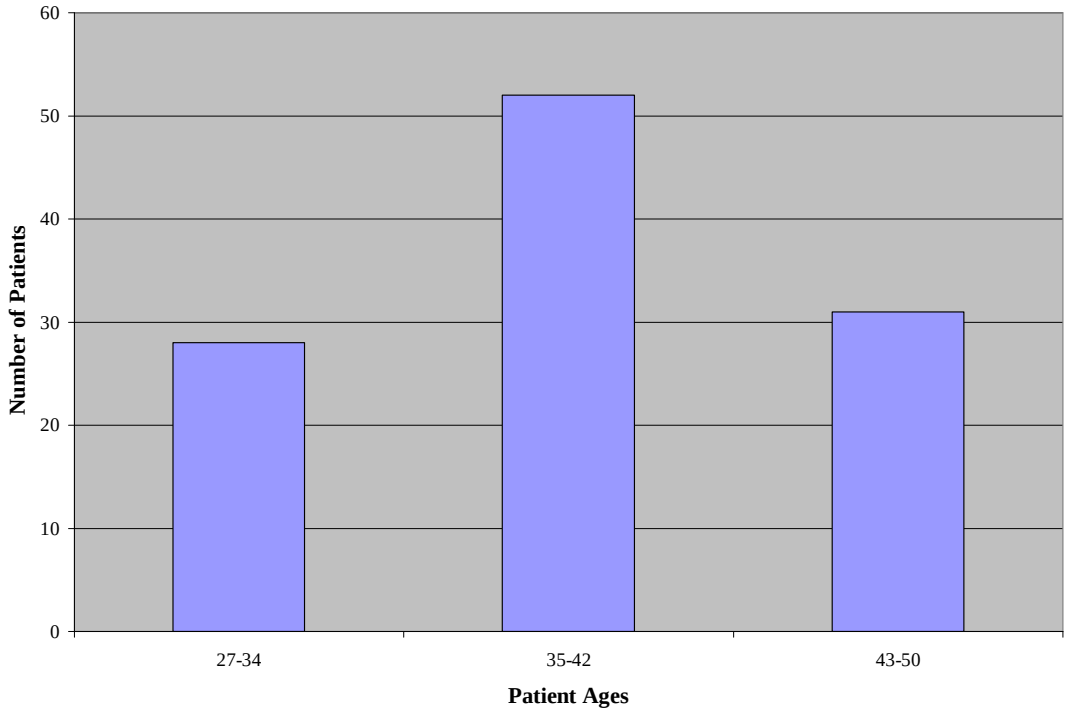


Figure 3.8: Age distribution amongst the patients (n = 111)

Age, however, had no significant influence on warfarin dosage in these patients (based on the linear models summarised in table 3.16 section 3.2.2.4). This is possibly due to the narrow range in age amongst our patients.

3.2.1.2 Concomitant Medication

Some of the patients were taking other medications, in addition to warfarin. These and the numbers of patients taking these drugs are listed in table 3.10.

Table 3.10: List and frequencies of the concomitant medications taken by the patients

Drug	Used for:	Interaction with Warfarin	Number of Patients (n = 111)
Ace Inhibitors	Cardiac	Unknown [2]	(16) 14%
Aldactone	Diuretic	Unknown [2]	(8) 7%
Amiloride	Cardiac	Unknown [2]	(1) 1%
Amitryptyline	Anti-depressant	Unknown [2]	(1) 1%
Aspirin	Anticoagulation	Depresses the concentration of prothrombin and plasma – increases bleeding time *[2]	(40) 36%
Beta Blockers	Cardiac	Unknown [3]	(22) 20%
Cordarone	Cardiac	Decreases warfarin metabolism [1]	(7) 6%
Digoxin	Cardiac	Unknown [2]	(19) 17%
Epanutin	Anti-epileptic	May increase anticoagulant effect of warfarin [4]	(1) 1%
Isoptin	Cardiac	Unknown [2]	(2) 2%
Lasix	Cardiac/Diuretic	Unknown [2]	(55) 50%
Moduretics	Diuretic	Unknown [2]	(12) 11%
Nifedipine	Cardiac	May increase prothrombin time [2*]	(2) 2%
Slow K	Cardiac (Replacement)	Unknown [2]	(50) 45%
Tegretol	Anti-epileptic	Increases warfarin metabolism [1]	(1) 1%

References: [1] Horton and Bushwick, 1999; [2] RX drug list: The internet drug index; [3] Heart Health; [4] NetDoctor

The box plots for the 1st eight medications may be seen in figure 3.10

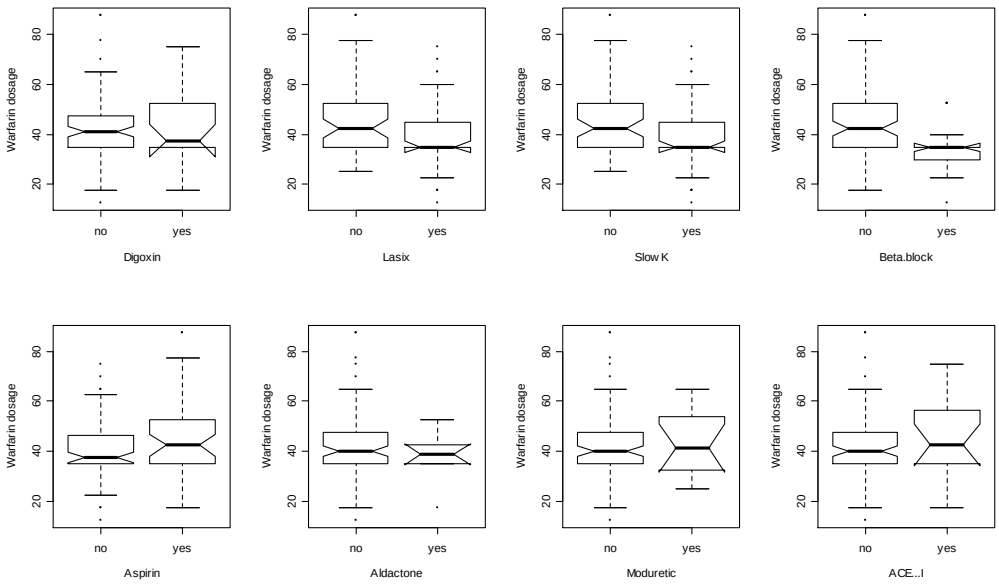
* Increases bleeding time and therefore assume lower doses of warfarin would be required

Amiloride, Amityptyline, Cordarone, Epanutin, Isoptin, Tegretol and Nifedipine were used by ≤ 6% of the patients and thus were excluded from further analysis.

3.2.1.2.1 Box Plots

Box plots were created to depict graphically the difference in warfarin dosage between patients who were taking a specific drug and those that were not. The box plots for the eight concomitant medications highlighted in table 3.10 may be seen in figure 3.9. On the X axis of each plot the box labelled “no” indicates that the patients in the box were not

taking the particular drug. The box labelled “yes” indicates that the patients in the box were taking the particular drug. The Y axis measures warfarin dosage in mg/week. In each box the confidence intervals are the arches in the centre of the box. The dark lines in the centre of the confidence intervals represent the median of the sample. The boxes around the confidence intervals are representative of the interquartile range of the samples. In each plot the horizontal lines extended from the plots with dotted lines represent the maximum and minimum values in the sample range. Any lines shown after the horizontal maximum or minimum lines represent outliers in the sample. In each plot, if the two confidence intervals overlap, this indicates that the particular drug of interest has no significant influence on warfarin dosage. Conversely, if the two confidence intervals do not overlap then the drug of interest does influence warfarin dosage.



On X-axis Yes = drug taken, No = drug not taken. Interquartile range = box; Confidence interval = arch in centre of box; Line in middle of the box = median of the sample. If the two confidence intervals overlap = no significant difference. If the two confidence intervals do not overlap = significant difference

Figure 3.9: Box plots depicting the relationship between the concomitant drugs and warfarin dosage

3.2.1.2.2 Wilcoxon Test

This test (described in section 2.2.7.2.1), was used to determine whether there were significant differences in warfarin maintenance dosage, between patients that were taking a particular concomitant drug to those that were not. Based on this test, beta blockers were the only drugs that significantly impacted warfarin maintenance dosage in the patients. The p-values of the different drugs according to the Wilcoxon test are listed in table 5.13 in Appendix K.

Based on the results from the Wilcoxon test and the box plots (summarised in table 3.11) I included aspirin, beta blockers, lasix and slow K in the linear models (shown in section 3.2.2.4). The linear models determine the influence of the particular variants on warfarin dosage, adjusting for the influence of these drugs and age.

Table 3.11: Summary of the results of the Wilcoxon test and box plots depicting the relationship between the concomitant drugs and warfarin dosage

Concomitant Drug	Wilcoxon Test	Box Plots
Ace Inhibitors	Insignificant	Insignificant
Aldactone	Insignificant	Insignificant
Aspirin	Insignificant	Slight increase in warfarin dosage
Beta Blockers	Significant	Significant decrease in warfarin dosage
Digoxin	Insignificant	Insignificant
Lasix	Insignificant	Slight decrease in warfarin dosage
Moduretics	Insignificant	Insignificant
Slow K	Insignificant	Slight decrease in warfarin dosage

The drugs that are highlighted in the table were those used for the linear models (section 3.2.2.4)

3.2.2 CYP2C9 and *VKORC1* variants

This section aims to determine the influence of the 14 variants on warfarin dosage. Table 3.12 lists the types of analyses that were used to determine the influence of these variants on warfarin dosage.

Table 3.12: List of analyses used to determine the influence of the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage

Analysis	Based on	Section
Kruskal-Wallis test	Genotype frequencies	3.2.2.1
Box plots	Genotype frequencies	3.2.2.2
Bar Graphs	Allele frequencies	3.2.2.3
Linear models	Genotype frequencies, adjusting for the influence of age, lasix, slow K, beta blockers and aspirin	3.2.2.4
Haplo.stats	Allele combinations	3.2.2.5

Of the three previously described *CYP2C9* variants that were observed in the patient and control samples, variant 1 (*CYP2C9*8*) decreases warfarin dosage *in vivo* but increases warfarin dosage *in vitro* (*CYP2C9* Allele Nomenclature Database). Variant 2's (*CYP2C9*9*) influence on warfarin dosage is unknown, and variant 3 (*CYP2C9*11*) decreases warfarin dosage (*CYP2C9* Allele Nomenclature Database).

3.2.2.1 Kruskal-Wallis Test

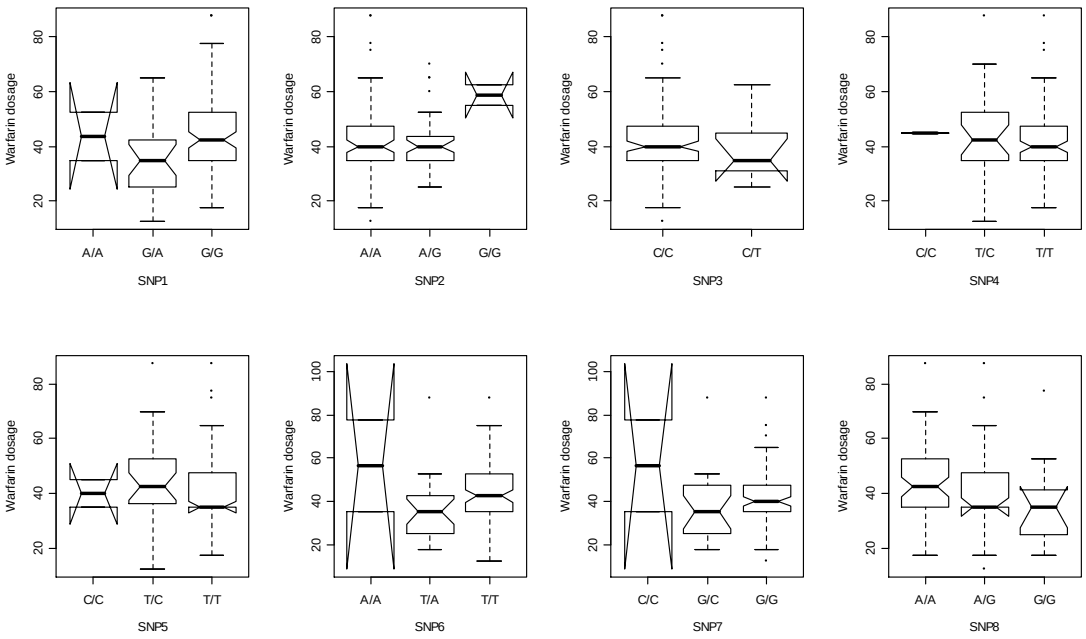
This test (described in section 2.2.7.2.2), was used to compare the median maintenance dosage between the three genotypes of the 14 *CYP2C9* and *VKORC1* variants. Only variants1 (*CYP2C9*8*) and 8 (*CYP2C9* 46028 A>G) significantly influence warfarin dosage. The chi-squared values, degrees of freedom and p-values for all 14 variants are shown in 5.14 in Appendix K.

3.2.2.2 Box Plots

Table 3.13 gives a list of the different genotypes for each of the 14 *CYP2C9* and *VKORC1* variants. Box plots were created for each of the 14 variants (shown in figures 3.10 and 3.11). Each plot shows the relationship between warfarin dosage and the three different genotypes of a particular variant. Some of these plots only have two genotypes due to the lack of patients with the homozygous variant genotype.

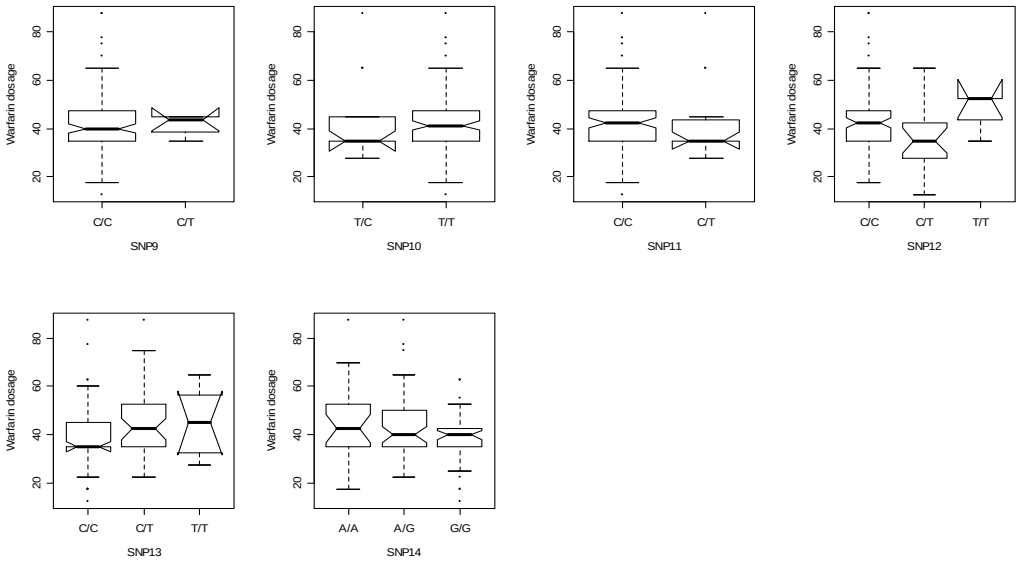
Table 3.13: List of the three possible genotypes for the 14 *CYP2C9* and *VKORC1* variants

Variant	Homozygous Normal	Heterozygous	Homozygous Variant
1	G/G	G/A	A/A
2	A/A	A/G	G/G
3	C/C	C/T	T/T
4	T/T	T/C	C/C
5	T/T	T/C	C/C
6	T/T	T/A	A/A
7	G/G	G/C	C/C
8	A/A	A/G	G/G
9	C/C	C/T	T/T
10	T/T	T/C	C/C
11	C/C	C/T	T/T
12	C/C	C/T	T/T
13	C/C	C/T	T/T
14	G/G	G/A	A/A



Interquartile range = box. Confidence interval = arch in centre of box. Line in middle of the box = median of the sample. If the two confidence intervals overlap = no significant difference. If the two confidence intervals do not overlap = significant difference

Figure 3.10: Box plots representing the influence of variants1 – 8 on warfarin dosage



Interquartile range = box; Confidence interval = arch in centre of box. Line in middle of the box = median of the sample. If the two confidence intervals overlap = no significant difference. If the two confidence intervals do not overlap = significant difference

Figure 3.11: Box plots representing the influence of variants 9 – 14 on warfarin dosage

Table 3.14 describes the impact of the two variant genotypes (heterozygotes and homozygotes for the variant) for each variant on warfarin dosage based on their box plots (figures 3.10 and 3.11).

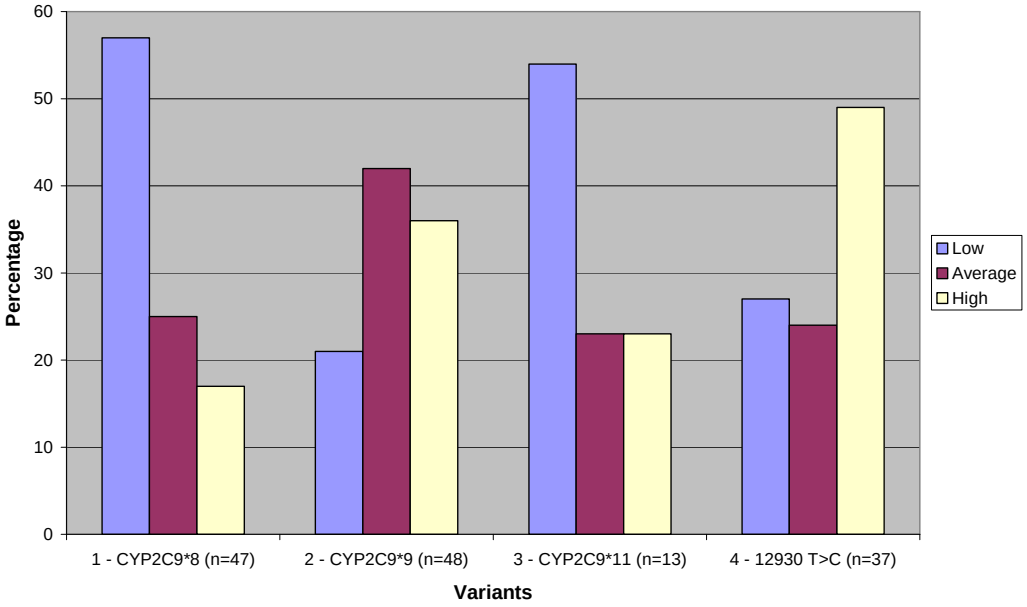
Table 3.14: A description of the impact of the two variant genotypes for the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage, based on the box plots

Variant	Heterozygous	Homozygous Variant
1	Decreased	Insignificant
2	Insignificant	Increased
3	Insignificant	Unknown*
4	Insignificant	Unknown*
5	Increased	Insignificant
6	Decreased	Unknown*
7	Insignificant	Unknown*
8	Decreased	Decreased
9	Insignificant	Unknown*
10	Decreased	Unknown*
11	Decreased	Unknown*
12	Decreased	Increased
13	Increased	Increased
14	Insignificant	Insignificant

*Unknown because I did not have any patients with the particular genotype or due to a standard error based on a small sample size.
Significant results are highlighted in the table

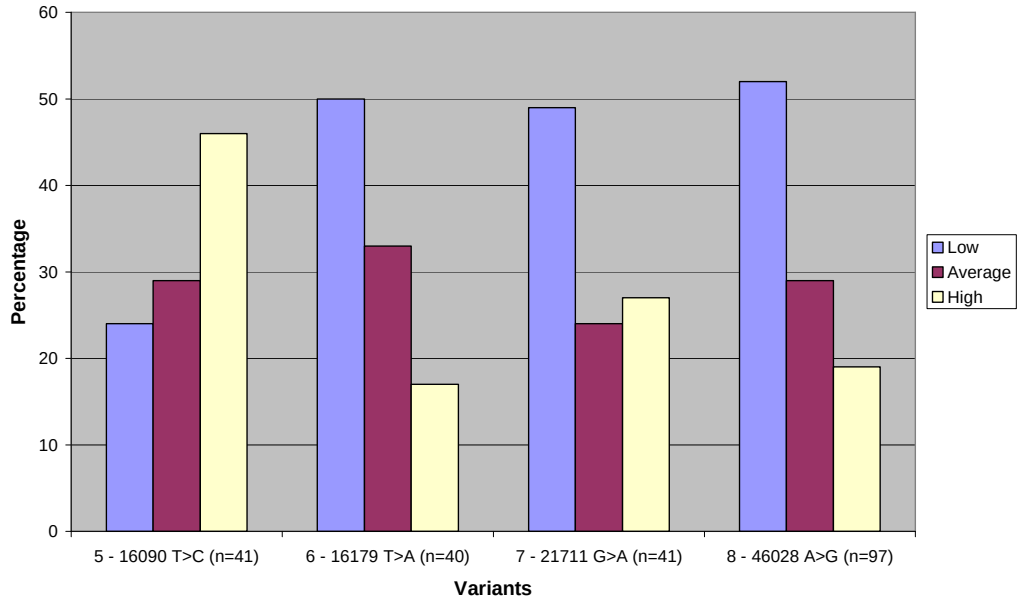
3.2.2.3 Bar Graphs

Bar graphs were used to compare the allele frequencies of the 14 *CYP2C9* and *VKORC1* variants between the different dosage groups (described in table 3.9, section 3.2). These may be seen in figures 3.12 – 3.15. Only the variant allele is represented in the graphs. The values in the brackets next to each variant name, represent the total number of variant alleles that were observed in the patient sample group (n = 110). The bars represent the percentage of the total number variant alleles that were observed in each dosage group.



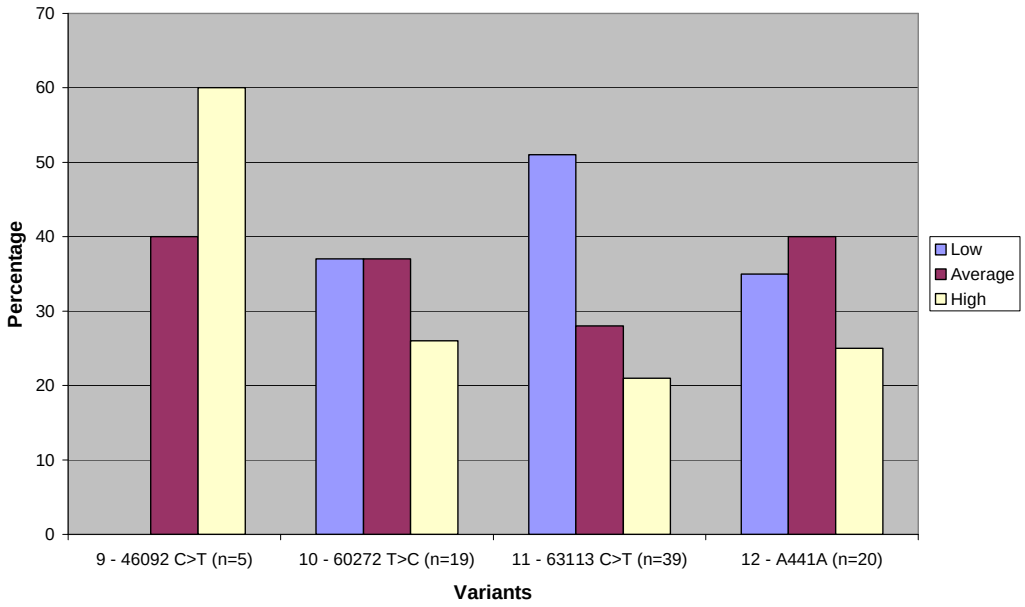
The numbers in the brackets on the X-axis are total number of variants alleles observed in the patients.
The bars = percentage of the total number of variant alleles observed in each dosage group.

Figure 3.12: Representation of the distribution of the variant alleles for variants 1 – 4 in each of the dosage groups



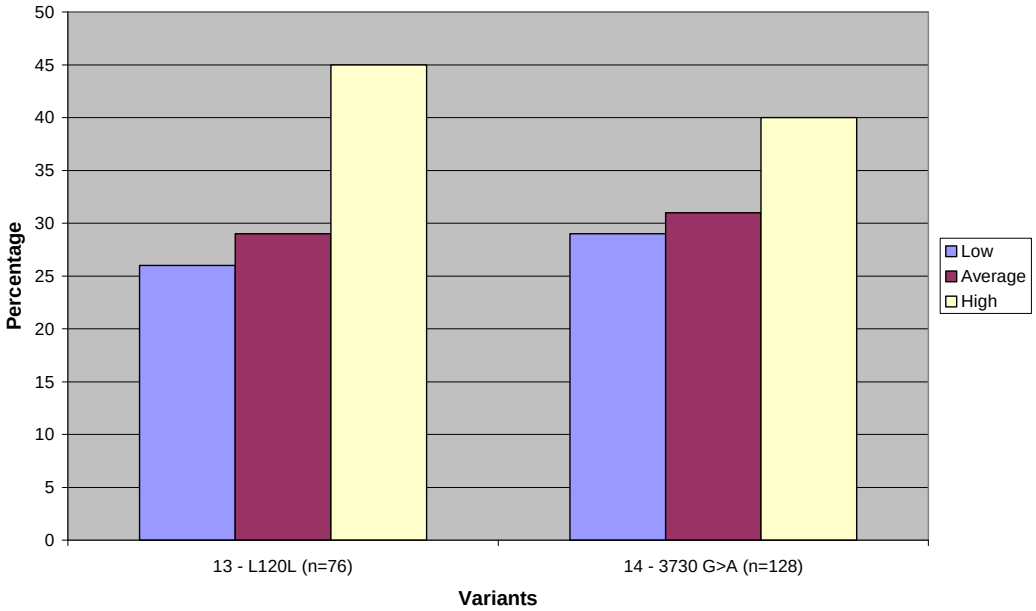
The numbers in the brackets on the X-axis are total number of variants alleles observed in the patients.
The bars = percentage of the total number of variant alleles observed in each dosage group.

Figure 3.13: Representation of the distribution of the variant alleles for variants 5 – 8 in each of the dosage groups



The numbers in the brackets on the X-axis are total number of variants alleles observed in the patients.
The bars = percentage of the total number of variant alleles observed in each dosage group.

Figure 3.14: Representation of the distribution of the variant alleles for variants 9 – 12 in each of the dosage groups



The numbers in the brackets on the X-axis are total number of variants alleles observed in the patients.
The bars = percentage of the total number of variant alleles observed in each dosage group.

Figure 3.15: Representation of the distribution of the variant alleles for variants 13 and 14 in each of the dosage groups

Table 3.15 summarises the results shown in all of the variant allele distribution graphs for each of the 14 *CYP2C9* and *VKORC1* variants and their proposed effects on warfarin dosage, based on their allele frequencies.

Table 3.15: Summary of the results shown in the allele frequency graphs for each of the 14 *CYP2C9* and *VKORC1* variants

Variant	Most Common In	Impact
1	Low Dose	Decreases metabolism
2	Average Dose	Unknown
3	Low Dose	Decreases metabolism
4	High Dose	Possibly increases metabolism
5	High Dose	Possibly increases metabolism
6	Low Dose	Possibly decreases metabolism
7	Low Dose	Possibly decreases metabolism
8	Low Dose	Possibly decreases metabolism
9	High Dose	Possibly increases metabolism
10	Low and Average Dose	Unknown
11	Low Dose	Possibly decreases metabolism
12	Average Dose	Unknown
13	High Dose	Possibly results in warfarin resistance
14	High Dose	Possibly results in warfarin resistance

Variants highlighted in lavender = may decrease warfarin dosage; Variants highlighted in yellow = may increase warfarin dosage; Variants that have not been highlighted may have no effect on warfarin dosage

In table 3.15, the variants highlighted in lavender are those that are most common in the patients on a low dose of warfarin. These are either known to decrease warfarin metabolism (variants 1 and 3 (*CYP2C9* Allele Nomenclature Database) or inferred to do so (because variants in the *CYP2C9* gene alter metabolism (Allabi et al., 2004)). The variants highlighted in yellow (in table 3.15), are most common in the patients on a high dose of warfarin. Thus are inferred to increase metabolism (variants 4, 5 and 9 found in the *CYP2C9* gene) or may result in warfarin resistance (variants 13 and 14 found in the *VKORC1* gene).

3.2.2.4 Linear Models

Linear models (described in section 2.2.7.2.2), were used to determine the level of impact the 14 variants have on warfarin dosage by adjusting for the four concomitant medications (identified in section 3.2.1) and age. Two linear models were created to determine the level of influence of the environmental factors: 1) Consisting of only the four concomitant drugs and 2) Consisting of the four concomitant drugs and age. To assess the influence of the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage, four types of models were created: 1) Involving separate models for each variant, including the environmental factors; 2) Consisting of all of the *CYP2C9* variants together and the environmental factors; 3) Consisting of the two *VKORC1* variants and the environmental factors and 4) Consisting of all 14 *CYP2C9* and *VKORC1* variants and the environmental factors.

All of these models may be seen in tables 5.15 – 5.33, Appendix K. Table 3.16 gives a summary of these results. In the table the estimated standard of the intercept represents the dosage of warfarin in mg/week that a patient would have if they were not taking lasix, slow K, beta blockers and aspirin, if their age was 0 years and they had all the wild type alleles for all 14 *CYP2C9* and *VKORC1* variants. The estimated standard values for each of the other coefficients show the influence that particular coefficient has on warfarin dosage compared to the intercept dosage. All the negative values decrease the intercept warfarin dosage, while the positive values increase the intercept warfarin dosage. The percentage variability is an estimate of the percentage the coefficients contribute towards warfarin dosage variability in the patients.

Table 3.16: Summary of the linear model results for all 14 *CYP2C9* and *VKORC1* variants and environmental factors

Coefficients	Estimated Standard (mg/week)	P-Value	% Variability
Intercept	51.63	1.03×10^{-8}	
Age	-0.27	0.2535	
Lasix	-3.20	0.5497	9.7
Slow K	1.28	0.7483	
Beta blockers	-8.03	0.0227	
Aspirin	3.70	0.1855	
Concomitant Drugs and Age			**10.6**
Variant 1 – N/V	-8.08	0.0092	5.9
Variant 1 – V/V	-1.06	0.9100	
Variant 2 – N/V	0.45	0.8750	3.1
Variant 2 – V/V	18.40	0.0630	
Variant 3 – N/V	-5.62	0.2570	1.2
Variant 4 – N/V	3.07	0.3220	1
Variant 4 – V/V	-4.27	0.7560	
Variant 5 – N/V	3.74	0.2140	2.2
Variant 5 – V/V	-8.64	0.3870	
Variant 6 – N/V	-8.47	0.0084	8
Variant 6 – V/V	13.43	0.1513	
Variant 7 – N/V	-4.40	0.1690	3.9
Variant 7 – V/V	14.531	0.1300	
Variant 8 – N/V	-3.93	0.1610	4.5
Variant 8 – V/V	-8.79	0.0370	
Variant 9 – N/V	-0.17	0.9810	0.1
Variant 10 – N/V	1.53	0.6920	0.2
Variant 11 – N/V	1.72	0.6490	0.3
Variant 12 – N/V	-5.68	0.0820	3.1
Variant 12 – V/V	4.22	0.5940	
All *CYP2C9* variants			**23.3**
Variant 13 – N/V	7.09	0.0100	6.9
Variant 13 – V/V	8.98	0.0750	
Variant 14 – N/V	5.81	0.044	5.3
Variant 14 – V/V	8.06	0.026	
Both *VKORC1* variants			**7.4**
All 14 *CYP2C9* and *VKORC1* variants			**34.7**
All 14 Variants and Environmental Factors			**45.3**

In the estimated standard column: negative values = decrease in warfarin dosage in mg/week, positive values = increase in warfarin dosage in mg/week. % Variability = the percentage of warfarin dosage variability a particular coefficient(s) account for in the patient sample.

From table 3.16 one can see that beta blockers and variants 1, 6 and 8 significantly decrease warfarin dosage by approximately eight mg/week. Variants 13 and 14 significantly increase warfarin dosage by seven mg/week and approximately six mg/week,

respectively. All of the environmental factors and the 14 *CYP2C9* and *VKORC1* variants, together, account for 45.3% of warfarin dosage variability in this patient sample, with the variants alone accounting for 34.7% and the environmental factors 10.6%

3.2.2.5 Haplo.stats Analysis

Allele combinations were created using Haplo.stats (described in section 2.2.7.2.2). The haplo.stats programme indirectly measured all the possible allele combinations in the patient sample group. All of these results may be seen in tables 5.34 - 5.43 in Appendix K. Table 3.17 shows only the allele combinations that significantly influence warfarin dosage. In the column labelled variants present: only the specific variants that are found in that particular allele combination are shown, all of the other alleles are therefore wild type. As with the linear models, the negative and positive values in the estimated standard column result in a decrease or increase in warfarin dosage as compared to the intercept, respectively.

Table 3.17: List of the allele combinations that significantly influence warfarin dosage in the patients, based on the haplo.stats results

Gene	Variants used	Allele combination number	Variants present	Allele combination Frequency	P-value	Estimated Standard (mg/week)
CYP2C9	1-12	6	3	0.02	0.000	- 1.98
	1-6	1	1	0.11	0.037	- 6.04
	3-8	3	8	0.16	0.026	- 5.89
	4-9	3	8	0.16	0.028	- 5.93
	5-10	4	8	0.16	0.012	- 6.15
	6-11	3	8	0.16	0.015	- 6.08
	7-12	3	8	0.07	0.051	- 6.21
	7-12	4	8, 12	0.10	0.032	- 6.34
VKORC1	13-14	2	13, 14	0.26	0.032	4.62
CYP2C9 and *VKORC1*	1-14	1	1, 8, 12, 14	0.03	0.000	- 7.43
	1-14	2	1, 8, 12	0.03	0.000	3.08
	1-14	9	2	0.10	0.021	6.11
	1-14	3	6, 7, 8	0.04	0.000	6.76
	1-14	8	8	0.03	0.000	3.80
	1-14	7	13, 14	0.13	0.000	11.59
	1-14		Rare	0.28	0.001	6.66

The allele combination number refers to the numbers shown in the haplo.stats results in tables 5:34-5.43 in appendix L. Variants present = only the variant alleles the rest are wild type.
In the estimated standard column: negative value = decrease in warfarin dosage in mg/week; Positive value = increase in warfarin dosage in mg/week

When I created allele combinations using only the *CYP2C9* variants, variants 1, 3, 8 and 12 were found to significantly decrease warfarin dosages. Variant 8 is the only variant present in a number of allele combinations and on average decreases warfarin dosage by 5.68 mg/week in approximately 12% of the patients. Variants 13 and 14 (found within the *VKORC1* gene) together increase warfarin dosage by approximately 8.11 mg/week. When I created allele combinations using both *CYP2C9* and *VKORC1* variants I note that most of the allele combinations result in an increase in warfarin dosage (with the exception of the first allele combination).

3.2.2.6 Summary of the influence of the 14 variants on warfarin dosage

Table 3.18 summarises all of the results obtained through the various analyses that were used to assess the influence of the different *CYP2C9* and *VKORC1* variants on warfarin dosage.

Table 3.18: Summary of the results of the different analyses used to determine the influence of the different *CYP2C9* and *VKORC1* variants on warfarin dosage

Variant	Kruskal-Wallis Test	Box Plots	Graphs	Linear Models	Haplo.stats Analysis
1	Significant	N/V ↓	↓	N/V ↓	↓
2	Insignificant	V/V ↑	Average dose	Insignificant	↑
3	Insignificant	Unknown	↓	Insignificant	↓
4	Insignificant	Unknown	↑	Insignificant	Insignificant
5	Insignificant	N/V ↑	↑	Insignificant	Insignificant
6	Insignificant	N/V ↓	↓	N/V ↓	Significant
7	Insignificant	Unknown	↓	Insignificant	Significant
8	Significant	N/V & V/V ↓	↓	N/V ↓	↓
9	Insignificant	Unknown	↑	Insignificant	Insignificant
10	Insignificant	N/V ↓	Low & Average Dose	Insignificant	Insignificant
11	Insignificant	N/V ↓	↓	Insignificant	Insignificant
12	Insignificant	N/V ↓, V/V ↑	Average dose	Insignificant	Significant
13	Insignificant	N/V & V/V ↑	↑	N/V ↑	↑
14	Insignificant	Insignificant	↑	N/V & V/V ↑	↑

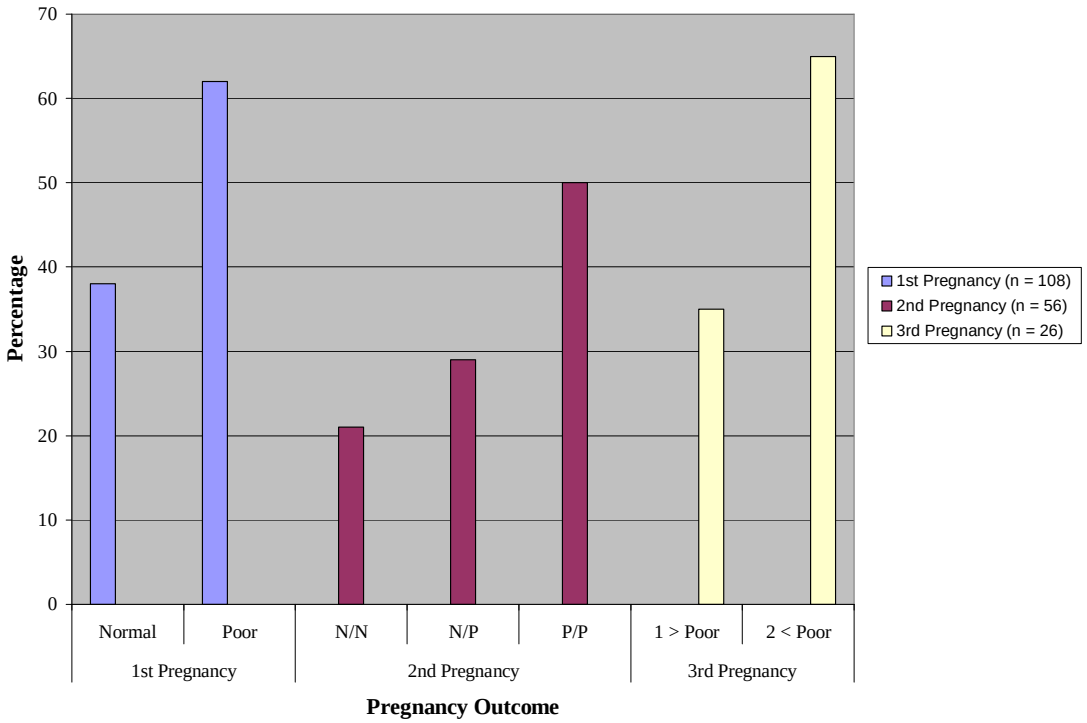
N/V = Heterozygotes, V/V = Homozygous for the variant. ↑ = Increased warfarin dosage; ↓ = Decreased warfarin dosage. Results highlighted in blue = decrease warfarin dosage; results highlighted in yellow = increase warfarin dosage; results highlighted in orange = significant results but the particular influence on warfarin dosage is either unknown or contradictory

In table 3.18 the blocks that are highlighted in orange represent significant results but the particular influence on warfarin dosage is either unknown or contradictory. The blocks highlighted in blue represent all the results that significantly decreased warfarin dosage. The blocks highlighted in yellow represent all the results that significantly increased

warfarin dosage. The significance of these results and the likely overall influence these variants have on warfarin dosage is discussed in section 4.2.

3.3 Pregnancy Outcome Analysis

The aim of this analysis was to determine whether or not the different *CYP2C9* and *VKORC1* variants influenced pregnancy outcome in patients taking warfarin. These analyses are based on the 14 *CYP2C9* and *VKORC1* variants (described in table 3.8, section 3.1.2.1) and the patients (n = 108). Bar graphs were created to show the number of poor and normal pregnancy outcomes in the patients after their first three pregnancies on warfarin (thereafter the sample sizes got too small, see table 2.1 in section 2.1.1). These graphs may be seen in figure 3.16. In the figure: N/N represents two normal outcomes, N/P represents one normal and one poor outcome and P/P represents two poor outcomes.



In the second pregnancy: N/N = two normal outcomes, N/P = one normal and one poor outcome, P/P = two poor outcomes. The number of pregnancy outcomes after the 3rd pregnancy was very small so I grouped the patients that had 1 ≥ poor outcomes (three good outcomes and (2 good outcomes and one poor outcome)) and the patients that had 2 ≤ poor outcomes ((two poor outcomes and one good outcome) and three poor outcomes)).

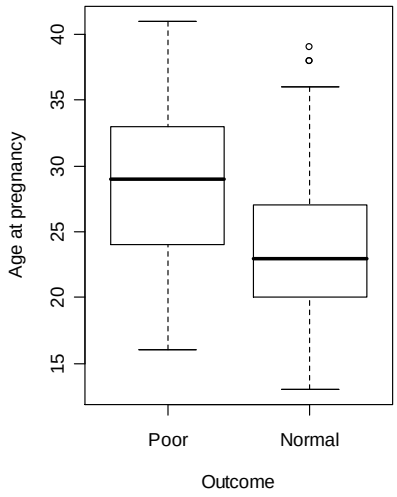
Figure 3.16: Illustration of the number of poor and normal pregnancy outcomes after the patients' 1st three pregnancies on warfarin

Figure 3.16 illustrates that there are more poor pregnancy outcomes than normal pregnancy outcomes after each pregnancy on warfarin. Environmental and genetic factors can influence pregnancy outcome. The only environmental factors that I was able to account for in this analysis were the age of the mother, whether or not they were on warfarin during pregnancy and whether or not they had changed to heparin for part of their pregnancy.

3.3.1 Environmental Factors

3.3.1.1 Age

Advanced maternal age is associated with an increased risk of having a child with an abnormality. These patients are similar as I observed an increase in poor pregnancy outcome with an increase in maternal age, despite the narrow range in age. Figure 3.17 shows the relationship between maternal age and pregnancy outcomes after the patients 1st pregnancy.



Interquartile range = box. Line in middle of the box = median of the sample.
On the X-axis poor = poor pregnancy outcome, normal = normal pregnancy outcome

Figure 3.17: Illustration of the effect of maternal age on pregnancy outcome

I used the generalised linear models and interaction models (described in sections 3.3.2.2 and 3.3.2.3) to assess the influence of age, number of pregnancies, heparin and warfarin on pregnancy outcome; and the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome accounting for the effects of age, number of pregnancies, heparin and warfarin. According to these models age significantly increased the risk of a poor pregnancy outcome.

3.3.1.2 Heparin

According to the generalised linear models and interaction models (described in sections 3.3.2.2 and 3.3.2.3), heparin significantly decreased the patients' risk of having a poor pregnancy outcome in the patient sample.

3.3.1.3 Warfarin

Warfarin taken during pregnancy significantly increased the patients' risk of having a poor pregnancy outcome. This can be seen in the generalised linear and interaction models (described in sections 3.3.2.2 and 3.3.2.3). Bar graphs were created to show the relationship between warfarin dosage and pregnancy outcome after the patients' 1st three pregnancies, using the three dosage groups (described in table 3.9, section 3.2). These are shown in figures 3.18 – 3.20. Our results are based on current warfarin dosage and not dosage during pregnancy. Warfarin dosage is usually increased during pregnancy; however, I expect that this increase is proportional to the patients' current warfarin dosage. Thus these results should reflect the situation during pregnancy.

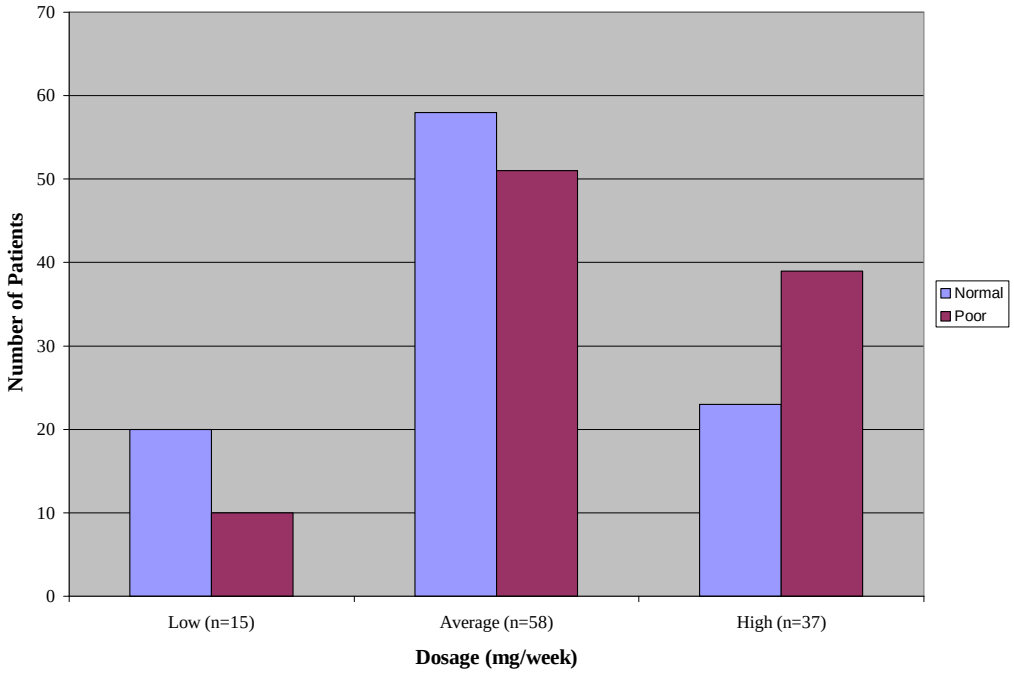
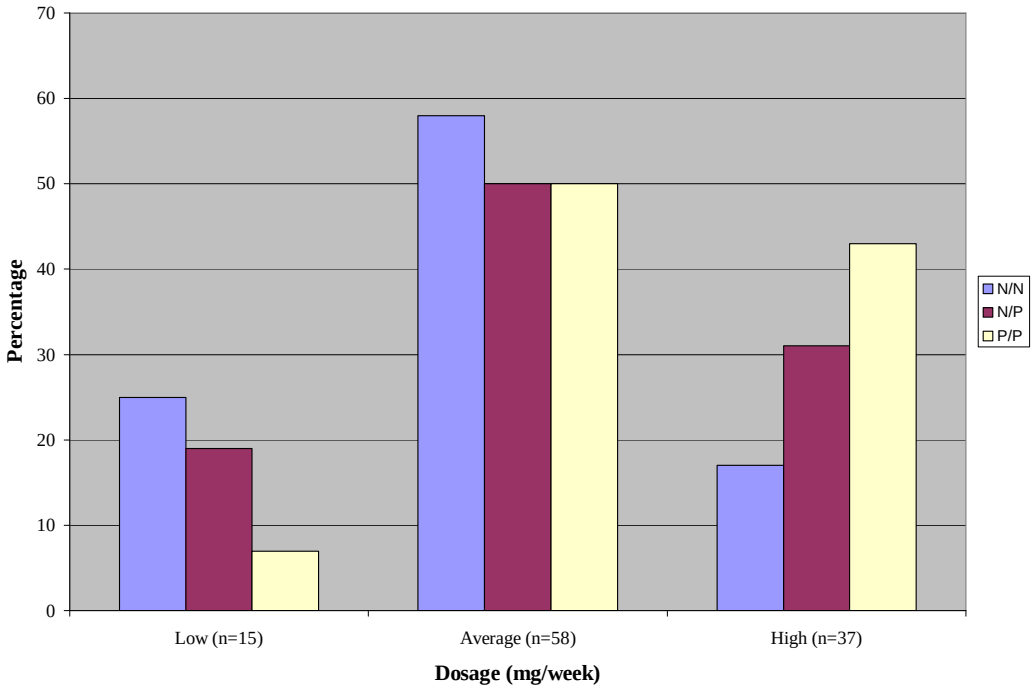
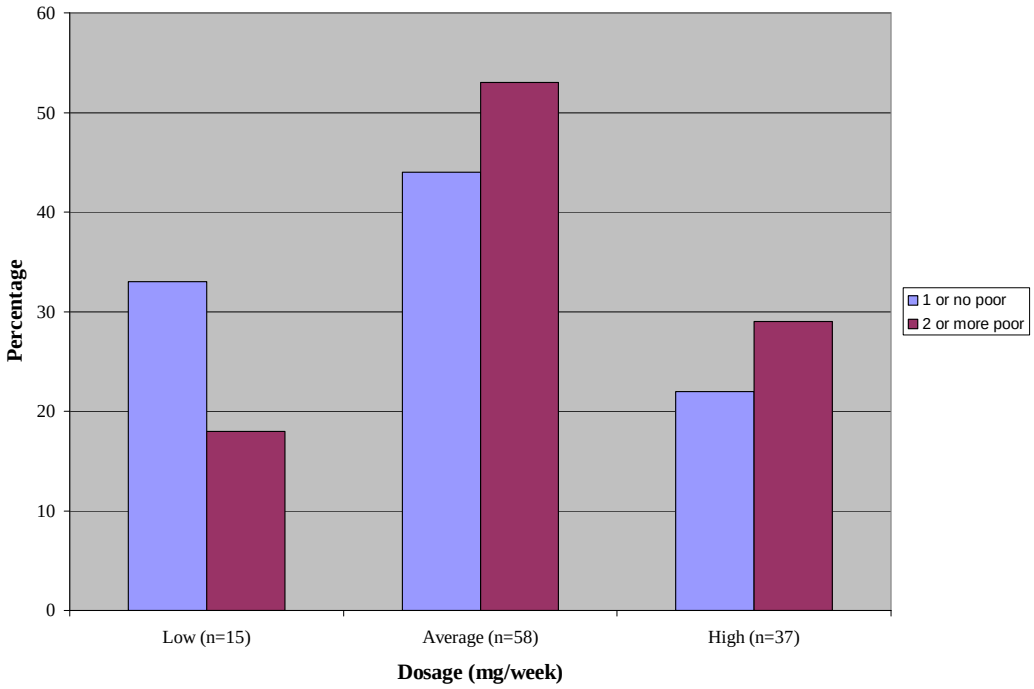


Figure 3.18: Influence of warfarin dosage on pregnancy outcome after one pregnancy



N/N =two normal outcomes, N/P = one normal and one poor outcome, P/P =two poor outcomes

Figure 3.19: Influence of warfarin dosage on pregnancy outcome after two pregnancies



Based on the sample size I grouped the pregnancy outcomes for patients that had one or no pregnancy outcomes (i.e. N/N/N and N/N/P) and patients that had two or more poor pregnancy outcomes (i.e. N/P/P and P/P/P)

Figure 3.20: Influence of warfarin dosage on pregnancy outcome after three pregnancies

All three of the figures showing the influence of warfarin dosage on pregnancy outcome (figures 3.18-3.20) show that there is an increase in poor pregnancy outcome with an increase in warfarin dosage.

3.3.2 *CYP2C9* and *VKORC1* variants

No statistical software is yet available to input different results (pregnancies) for a single factor (patient). Thus all of the analyses used to determine the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome are based on the pregnancy outcomes after the patients' 1st pregnancies. Table 3.19 summarises the different analyses used.

Table 3.19: Summary of all of the analyses used to determine the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome

Description	Based on	Section
Bar Graphs	Genotypes	3.3.2.1
Generalised Linear Models	Genotypes	3.3.2.2
Interaction Models	Alleles	3.3.2.3
Haplo.stats Analysis	Allele combinations	3.3.2.4

3.3.2.1 Bar Graphs

Bar graphs were created to show the frequency of poor and normal pregnancy outcomes for each of the 14 variants for each genotype (shown in figures 5.1 and 5.2 in Appendix L). Most of the results for these graphs were insignificant. Individuals with variants 9, 10, 11 and 13, however, had more normal pregnancy outcomes than poor. The lack of statistical significance of these results may be attributed to the fact that small numbers of patients that were heterozygous or homozygous for the variant allele were analysed. In addition these graphs determine the influence of these variants on pregnancy outcome independent of warfarin taken during pregnancy and it would seem unlikely that they influence pregnancy outcome outside of their effect on warfarin dosage.

3.3.2.2 Generalised Linear Models

Generalised linear models (described in section 2.2.7.3.2) were used to determine the effects of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome, accounting for the effects of age, number of pregnancies, warfarin and heparin treatment. These models may be seen in tables 5.44 - 5.59 in Appendix L. Table 3.20 summarises these results. In the table the variants highlighted in yellow appear to increase the risk of a poor pregnancy

outcome. The variants highlighted in orange decrease the risk of a poor pregnancy outcome.

Table 3.20: Summary of the effects of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome according to the generalised linear models

Variant	Influence on pregnancy outcome
1	Insignificant
2	Homozygotes increase risk of a poor pregnancy outcome
3	Insignificant
4	Insignificant
5	Insignificant
6	Insignificant
7	Insignificant
8	Insignificant
9	Insignificant
10	Insignificant
11	Insignificant
12	Insignificant
13	Heterozygotes and homozygotes decrease risk of having a poor pregnancy outcome
14	Insignificant

The variants that are highlighted in the table significantly influence pregnancy outcome. Variant 2 highlighted in yellow = increases the risk of poor pregnancy outcome, Variant 13, highlighted in orange = decreases the risk of a poor pregnancy outcome

3.3.2.3 Interaction Models

These models (described in section 2.2.7.3.2), show the effect of the different variants on pregnancy outcome when they interact with warfarin. I was unable to get results for variants 6, 9, 10, 11 and 13, as R was unable to produce models for these variants, the reasons for which are unknown. The models for the other variants may be seen in tables 5.60 - 5.68 in Appendix L. Table 3.21 summarises these results. In the table the variants highlighted in yellow appear to increase the risk of a poor pregnancy outcome. The variants highlighted in orange decrease the risk of a poor pregnancy outcome.

Table 3.21: Summary of the effects of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome according to the interaction models

Variant	Influence on pregnancy outcome independent of warfarin	Influence on pregnancy outcome when interacting with warfarin
1	Insignificant	Insignificant
2	Increased risk of a poor pregnancy outcome	Insignificant
3	Increased risk of a poor pregnancy outcome	Decreased risk of a poor pregnancy outcome
4	Insignificant	Insignificant
5	Insignificant	Insignificant
6	Unknown	Unknown
7	Insignificant	Insignificant
8	Insignificant	Insignificant
9	Unknown	Unknown
10	Unknown	Unknown
11	Unknown	Unknown
12	Insignificant	Insignificant
13	Unknown	Unknown
14	Decreased risk of a poor pregnancy outcome	Insignificant

Variants highlighted in yellow = significantly increase the risk of a poor pregnancy outcome. Variants highlighted in orange = significantly decrease the risk of a poor pregnancy outcome.

3.3.2.4 Haplo.stats Analysis

Haplo.stats analyses (described in section 2.2.7.3.2), were carried out to determine if there were any allele combinations that influenced pregnancy outcome in the patients on and off warfarin during pregnancy. Table 3.22 summarises the number of first pregnancies and their outcomes used in these analyses.

Table 3.22: Summary of the number of 1st pregnancies used for the haplo.stats analyses

Pregnancies	Normal Outcomes	Poor Outcomes	Total
On warfarin	30	38	68
Off warfarin	37	4	41

All the haplo.stats results may be seen in tables 5.69 – 5.88 in Appendix L. Onlyone*VKORC1* allele combination (in which variant 14 is present) significantly increased the risk of having a poor pregnancy outcome in the patients that were not on warfarin during pregnancy. This result may be an artefact due to the small number of pregnancies off warfarin and the uneven distribution of normal and poor outcomes.

3.3.2.5 Summary of the influence of the 14 variants on pregnancy outcome

Table 3.23 summarises all of the results obtained through the various analyses that were used to determine the influence of the different *CYP2C9* and *VKORC1* variants on pregnancy outcome. In the table the variants highlighted in yellow appear to increase the risk of a poor pregnancy outcome. The variants highlighted in orange decrease the risk of a poor pregnancy outcome.

Table 3.23: Summary of the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome

Variant	Bar Graphs	Generalised Linear Models	Interaction Models	Haplo.stats Analysis
1	Insignificant	Insignificant	Insignificant	Insignificant
2	Insignificant	V/V ↑ risk of poor outcome	Insignificant	Insignificant
3	Insignificant	Insignificant	↓ risk of poor outcome	Insignificant
4	Insignificant	Insignificant	Insignificant	Insignificant
5	Insignificant	Insignificant	Insignificant	Insignificant
6	Insignificant	Insignificant	Insignificant	Insignificant
7	Insignificant	Insignificant	Insignificant	Insignificant
8	Insignificant	Insignificant	Insignificant	Insignificant
9	More normal pregnancy outcomes than poor	Insignificant	Insignificant	Insignificant
10	More normal pregnancy outcomes than poor	Insignificant	Insignificant	Insignificant
11	More normal pregnancy outcomes than poor	Insignificant	Insignificant	Insignificant
12	Insignificant	Insignificant	Insignificant	Insignificant
13	More normal pregnancy outcomes than poor	N/V & V/V ↓ risk of poor outcome	Insignificant	Insignificant
14	Insignificant	Insignificant	Insignificant	Insignificant

Results highlighted in orange = significantly decrease the risk of a poor pregnancy outcome. Results highlighted in yellow = significantly increase the risk of a poor pregnancy outcome.

Most of the results of the different analyses used (shown in table 3.23) were statistically insignificant. This could be as a result of the small number of patients with a particular variant genotype. All the pregnancy analysis results are discussed in section 4.3.

4 DISCUSSION

This chapter summarises and discusses the results presented in section 3. Section 4.1 discusses the known and novel *CYP2C9* and *VKORC1* variants I observed in the patient and control samples. Section 4.2 discusses the implications of these variants and a small subset of environmental factors for warfarin dosage; and the importance of these variants with respect to pharmacogenetic testing (for warfarin administration). Section 4.3 discusses the implications of these variants and a small subset of environmental factors with respect to pregnancy outcome when warfarin is taken during pregnancy; and the importance of these in genetic counselling (concerned with the risks involved in taking warfarin during pregnancy). Section 4.4 discusses the limitations of this and similar projects.

4.1 Variant Analysis

Section 4.1.1 deals with the *CYP2C9* variants. Section 4.1.2 deals with the *VKORC1* variants. Section 4.1.3 discusses the differences in genetic variation between populations of African origin. Section 4.1.4 discusses future studies that may be implemented based on the observed *CYP2C9* and *VKORC1* variants in the SA black population.

4.1.1 *CYP2C9* Variants

4.1.1.1 Previously Described Variants

Of the 30 described *CYP2C9* variants (listed in table 1.2 in section 1.3) six are reported to be common in populations of African origin (African-American, African Pygmies or Beninese) (Dickmann et al., 2001; Allabi et al., 2003; Blaisdell et al., 2004). Of these six

variants, five were observed in the SA population. Of the remaining 24 variants (found in populations of non-African origin, listed in table 1.2 in section 1.3), one was observed in the SA population. Table 4.1 gives a list of the observed *CYP2C9* variants in the SA population and a comparison of the allele frequencies at which they have been previously reported to those observed in the SA black population in the current study.

Table 4.1: List and allele frequencies of the previously described *CYP2C9* variants that were observed in the SA population

Variant	Previously Reported Population	Reported Allele Frequency	Observed Allele Frequency)*
*CYP2C9*3* (n) frequency	Caucasian Asian/African-American	0.2-0.3 0.05 [4]	(2/426) 0.005
*CYP2C9*5* (n) frequency	African – American	(5/240) 0.02 [1]	(2/426) 0.005
*CYP2C9*6* (n) frequency	African – American	(10/158) 0.06 [2]	(1/426) 0.002
*CYP2C9*7* (n) frequency	African – American and African Pygmies	(1/48) 0.02 [3]	(0/426) 0
*CYP2C9*8* (n) frequency	African – American and African Pygmies	(2/44) 0.05 [3]	(46/426) 0.11
*CYP2C9*9* (n) frequency	African – American and African Pygmies	(8/48) 0.16 [3]	(66/426) 0.15
*CYP2C9*11* (n) frequency	African – American and African Pygmies	(1/48) 0.02 [3]	(17/426) 0.04

* Frequency of the combined patients and controls in this study.

References: [1] Dickmann et al., 2001, [2]; Allabi et al., 2003; [3] Blaisdell et al., 2004, [4] Kimball Genetics Website

Variants *CYP2C9*5*, *CYP2C9*6* and *CYP2C9*7* were observed, in the SA black population, at lower frequencies than those reported in other populations of African origin. Variants *CYP2C9*8* and *CYP2C9*11* were observed, in the SA population, at frequencies twice as high as those reported in African-American and African Pygmies, suggesting that

this variant is of African origin. The exact region of origin, however, is unknown. Variant *CYP2C9*9* is the only variant that was observed at a similar frequency to those previously reported. The allele frequencies that have been previously reported were observed in small sample sizes and may therefore not be representative.

4.1.1.2 Novel Variants

Twenty-seven new *CYP2C9* variants were identified within the patient and control sample groups. Table 4.2 lists these variants and how they affect mephenytoin 4-hydroxylase (the drug metabolising enzyme encoded by *CYP2C9*).

Table 4.2: List of the 27 novel *CYP2C9* variants and their affect on mephenytoin 4-hydroxylase

Single Nucleotide Polymorphism (SNP)	Expected Amino Acid Change	Other sequence changes
12803 A>G	I42V	Missense
12930 T>C (4)	-	Possible Splice Site
15906 A>G	I74V	Missense
15913 T>A	V76Q	Missense
16060 T>C	-	Unknown
16090 T>C (5)	-	Possible Splice Site
16094 T>C	-	Possible Splice Site
16179 T>A (6)	-	Unknown
16247 G>T	T130T	Silent
21711 G>C (7)	-	Possible Splice Site
21748 G>A	-	Unknown
46028 A>G (8)	-	Possible Splice Site
46092 C>T (9)	-	Possible Splice Site
55198 T>C	I327T	Missense
60175 A>G	-	Possible Splice Site
60225 T>A	-	Unknown
60272 T>C (10)	-	Unknown
60318 C>T	-	Unknown
60328 A>G	-	Unknown
62875 C>T (11)	A441A	Silent
62941 C>T	D463D	Silent
62977 A>T	G465G	Silent
63092 C>T	-	Unknown
63113 C>T (12)	-	Unknown
63143 C>G	-	Possible Splice Site
63169 G>A	-	Unknown
63180 C>T	-	Possible Splice Site

The variants that are highlighted are those observed at a frequency of ≥ 0.02, thus useful for further analysis. SNP = single nucleotide change in the DNA strand. The numbers in the brackets behind each of the highlighted variants are the identity numbers that were used for each analysis (as seen in table 3.8 in section 3.1.2.1).

In table 4.2 the novel variants that have been highlighted were those observed at a frequency of 0.02 or above, and were used for further analysis together with five of the previously described variants (3 *CYP2C9* and two *VKORC1*) (described in sections 3.1, 3.2 and 3.3).

Isoleucine (I) and Valine (V) are both part of the aliphatic amino acid group, with non-polar side chains, (Mathews et al., 2000). Glutamine (Q) is an acidic amino acid with an

uncharged polar side chain (Mathews et al., 2000). Threonine (T) also has an uncharged polar side chain with a hydroxyl group present (Mathews et al., 2000). Thus, it would be expected that V76Q (15913 T>A) and I327T (55198 T>C) mutations to alter the CYP2C9 protein structure more significantly than the I42V (12803 A>G) and I74V (15906 A>G) substitutions. This hypothesis could not be confirmed because the numbers of patient and control samples with these variant alleles were too small (i.e. observed at an allele frequency of ≤ 0.02) thus these variants were not used for further analyses.

Five of the nine possible splice site mutations were used for the variant, warfarin dosage and pregnancy outcome analyses. Although I was able to assess their influence on warfarin dosage (discussed in section 4.2.2), more studies would need to be carried out on all the possible splice site mutations to assess whether they do alter the splicing of the gene. Similarly functional analyses could be carried out on all 27 novel variants to determine their effect on the structure and function of this drug metabolising enzyme.

4.1.2 *VKORC1* Variants

Of the 11 previously described *VKORC1* variants I screened, I observed three in the patient and control samples. These three variants were previously identified in Caucasian populations from the United Kingdom (V66M) and Italy (L120L and 3730 G>A), but have not been described in populations of African origin. Table 4.3 compares the allele frequencies at which these variants were reported to those observed in the SA black population.

Table 4.3: List of three observed *VKORC1* variants and their allele frequencies as reported and observed in the patient and control samples

Variant	Reported Allele Frequency	Observed Allele Frequency in the patient and control samples
V66M (n) frequency	(1/1640) 0.0007 [2]	(2/256) 0.007
L120L (n) frequency	(2/294) 0.007 [1]	(97/424) 0.23
3730 G>A (n) frequency	(102/294) 0.35 [1]	(184/426) 0.43

References: [1] D'Andrea et al., 2005 (Caucasian – Italian), [2] Harrington et al., 2005 (Caucasian – UK)

The L120L *VKORC1* variant was observed at a statistically significantly higher allele frequency in the SA black population than the Caucasian population (shown in table 4.3). The other two *VKORC1* variants (V66M and 3730 G>A) were also at a higher allele frequency in the SA black population than those reported in the Caucasian populations (shown in table 4.3), however this is not statistically significant.

4.1.3 Genetic variation amongst populations of African origin

Early studies in genetic diversity showed that most genetic diversity was found between individuals rather than between populations or continents (Reviewed in: Serre and Paabo, 2004). Recent studies suggest that human genetic diversity is organised in continental clades (Reviewed in: Serre and Paabo, 2004). It is well known that human genetic diversity for many traits is higher in sub-Saharan Africa than in other geographic regions (Releford, 2001; reviewed in: Tishkoff et al., 2004). This diversity is of major medical relevance as individuals from different origins respond differently to medical treatments (Reviewed in: Serre and Paabo, 2004; reviewed in: Tishkoff et al., 2004; Bamshad, 2005).

Some of the *CYP2C9* and *VKORC1* variants I observed in the SA black population have been previously described in Caucasian, Asian, African-American and African Pygmy populations. African-American populations have been reported to possess 20% European heritage (The African American MS Genetics Project). African Pygmies are populations that originate in central Africa (Encyclopaedia Britannica: Pygmy). I expected to find similar variation in the SA black population and the African-American and African Pygmy populations rather than between the SA black population and Caucasian and Asian populations. Within the *CYP2C9* gene, only two of the six previously described variants showed statistically significant differences between the African-American/African-Pygmy populations and the SA black population. In addition I observed 27 novel variants within this gene amongst the SA black population.

None of the *VKORC1* variants I observed in the SA black population have been previously described in a population of African origin. No novel variants were observed within this gene amongst the SA black population. The human *VKORC1* gene has high homology with 38 other species (Ensembl). Thus this homology and the lack of genetic diversity seen within this gene amongst the SA population suggest that this gene has been highly conserved. This conservation may explain the significant influence variants within this gene have on warfarin dosage.

4.1.4 Future Studies involving the observed *CYP2C9* and *VKORC1* variants

The genetic variation shown within the SA black population compared to other populations highlights the importance of studying this population and designing tests specific to this population. Only nine of the 27 novel *CYP2C9* variants (highlighted in table 4.2 in section 4.1.1.2) were observed at a frequency of 0.02 or above and could be used for further

analyses. Although tests are based on the most frequently observed variants within populations, it would be important to understand the function of the other variants as they may affect dosage. Thus, further studies would need to be carried out on the other 18 variants to assess their influence on warfarin dosage and pregnancy outcome. A test aimed at rapidly detecting these novel variants would need to be designed. I could then screen a larger number of patients for these variants. I would then be able to correlate these variants to warfarin dosage and pregnancy outcome. Other studies aimed at determining the frequency of these novel and previously described *CYP2C9* and *VKORC1* variants in other SA populations such as Caucasian, Indian and mixed ancestry could be carried out. Pharmacogenetic testing altered for screening South African populations and genetic counselling for pregnant mothers on warfarin will be discussed in sections 4.2.3 and 4.3.3, respectively.

4.2 Warfarin Dosage Variability Analysis

This section is divided into three sections. Section 4.2.1 discusses the influence of the environmental factors (age and concomitant medication) on warfarin dosage. Section 4.2.2 discusses the influence of the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage. Section 4.2.3 discusses the implications of using these environmental factors and the 14 *CYP2C9* and *VKORC1* variants for pre-administration pharmacogenetic testing of SA black patients for warfarin administration.

4.2.1 Influence of the environmental factors on warfarin dosage

4.2.1.1 Age

Age has an inverse relationship with warfarin dosage (older patients require lower doses of warfarin to maintain adequate anticoagulation) (Horton and Bushwick, 1999). The patients' ages in this study ranged from 27-50 with the median being 40. Although age showed a decrease in warfarin dosage compared to the intercept's estimated standard in the linear models (summarised in table 3.13 in section 3.2.2.4) it was insignificant. This insignificance may be attributed to the narrow range in age amongst the patients. Thus the influence age has on warfarin dosage may only be seen at extreme differences in age.

4.2.1.2 Concomitant Medication

Of the 15 concomitant medications identified in the patients (listed in table 3.9 in section 3.2.1.2) seven were excluded from further analysis because the numbers of patients taking those medications were too small. The influence of the eight remaining medications on warfarin dosage was assessed using box plots and a Wilcoxon test. Based on these results four medications (lasix, slow K, beta blockers and aspirin) were selected for further analysis using the linear models. The influence of these four medications on warfarin dosage in the patients are summarised in table 4.4.

Table 4.4: Summary of the influence of the lasix, slow K, beta blockers and aspirin on warfarin dosage based on the box plots, Wilcoxon test and linear models

Drug	Box Plots	Wilcoxon Test	Linear Models
Lasix	Decreases warfarin dosage slightly	Insignificant	Insignificant
Slow K	Decreases warfarin dosage slightly	Insignificant	Insignificant
Beta Blockers	Decreases warfarin dosage	Significant	Decreases warfarin dosage
Aspirin	Increases warfarin dosage slightly	Insignificant	Insignificant

The results that are highlighted in the table showed a significant influence on warfarin dosage

According to the box plots (summarised in table 4.4) the patients taking lasix and slow K showed a slight decreased in warfarin dosage compared to the patients that were not taking these drugs. The influence of these drugs on warfarin, according to the Wilcoxon test and linear models were insignificant. Aspirin is used as an anticoagulant (amongst other things). Thus I would expect that patients taking aspirin and warfarin would require lower doses of warfarin to maintain adequate anticoagulation. In our study, however, the patients that were taking aspirin and warfarin had increased doses of warfarin compared to the patients that were not taking aspirin. These results, like that of lasix and slow K were only observed minimally in the box plots and were not repeated in the Wilcoxon test or linear models, and may not be significant.

Beta blockers are the only concomitant medications that significantly decreased warfarin dosage in the patients in all of our analyses. This decrease, according to the linear models, can be from 6.59 mg/week to 11.74 mg/week, with an average of 8.00 mg/week. This finding has been previously unreported in drugs that interact with warfarin (RX Drug Index). Patients taking warfarin for a cardiac defect are likely to be taking beta blockers. Thus the knowledge that beta blockers influence warfarin dosage is important. These

results imply that one could reduce the risk of haemorrhagic complications in a patient taking warfarin and beta blockers by reducing their warfarin dosage by approximately 8mg/week.

4.2.2 Influence of 14 *CYP2C9* and *VKORC1* variants on warfarin dosage

The influence that the 14 observed *CYP2C9* and *VKORC1* variants have on warfarin dosage was analysed in sections 3.2.2.1 – 3.2.2.5 (summarised in table 3.14 in section 3.2.2.6). Only the variants that showed significance in two or more of the analyses presented in section 3.2.2 were considered to have a significant influence on warfarin dosage. Table 4.5 gives a summary of the hypothesised overall influence of the variants on warfarin dosage.

Table 4.5: Summary of the hypothesised influence of the 14 *CYP2C9* and *VKORC1* variants on warfarin dosage

Variant ID	Variant	Type of Mutation	Influence
1	*CYP2C9*8*	Missense	Decreases dosage
2	*CYP2C9*9*	Missense	Increases dosage
3	*CYP2C9*11*	Missense	Decreases dosage
4	12930 T>C	Splice Site	None observed
5	16090 T>C	Splice Site	Increases dosage
6	16179 T>A	Unknown	Decreases dosage
7	21711 G>C	Splice Site	Decreases dosage
8	46028 A>G	Splice Site	Decreases dosage
9	46092 C>T	Splice Site	None observed
10	60272 T>C	Unknown	None observed
11	62875 C>T	Silent	Decreases dosage
12	63113 C>T	Unknown	Contradictory
13	*VKORC1* L120L	Silent	Increases dosage
14	*VKORC1* 3730 G>A	3'UTR SNP	Increases dosage

The variants highlighted in blue appear to decrease warfarin dosage. The variants highlighted in yellow appear to increase warfarin dosage. The variants that are not highlighted may have no influence on warfarin dosage.

In table 4.5, all of the variants that are highlighted in blue are known (in the case of *CY92C9*11*) or hypothesised to decrease warfarin dosage. The variants that are

highlighted in yellow are those that are hypothesised to increase warfarin dosage. The described *CYP2C9*8* variant's influence on warfarin dosage has been contradictory (decreased *in vivo* but increased *in vitro* (*CYP2C9* Allele Nomenclature Database)), appears to decrease warfarin dosage in the patients, consistent with *in vivo* studies. The described *CYP2C9*9* variant's influence on warfarin dosage is unknown (*CYP2C9* Allele Nomenclature Database), appears to increase warfarin dosage in the patients. The described *CYP2C9*11* variant, which is known to decrease warfarin dosage (*CYP2C9* Allele Nomenclature Database), also decreases warfarin dosage in the patients, confirming the previously observed effect.

Of the nine novel *CYP2C9* variants I observed, four appear to decrease warfarin dosage, 1 appears to increase warfarin dosage, while the remaining four appear to have no or a contradictory effect on warfarin dosage. Mutations within the *CYP2C9* gene alter warfarin metabolism and result in patients requiring altered doses of warfarin to maintain adequate anticoagulation (Allabi et al., 2004). Thus the novel *CYP2C9* variants that appear to alter warfarin dosage may do so as a result of an increase (variant 5) or decrease (variants 6, 7, 8 and 11) in the metabolism of warfarin.

The *VKORC1* L120L variant (variant 13) has been described as having no influence on warfarin dosage (D'Andrea et al., 2005). In the patients, however, I observed an increase in warfarin dosage amongst patients with this variant. This result is very surprising considering that this variant is a synonymous mutation. Further studies concerning this variant would need to be carried out to determine how its presence results in an increase in warfarin dosage. The *VKORC1* 3730 G>A variant's (variant 14) influence on warfarin dosage has been described as unknown (D'Andrea et al., 2005), however, it appears to

increase warfarin dosage in the patients I studied. Mutations within the *VKORC1* gene mostly result in warfarin resistance (Rost et al., 2004) and this is supported by the evidence from these two variants which may also result in warfarin resistance.

The linear models (described in section 3.2.2.4) and haplo.stats analysis (described in section 3.2.2.5) show that variants 1, 3, 6 and 8, on average, decrease warfarin dosages by seven, two, eight and seven mg/week, respectively. Variants 13 and 14 increase warfarin dosages on average by five mg/week. These results suggest that one would alter the dosage of warfarin for a patient who has one of these variant alleles accordingly. However, most of the patients have more than one variant allele. The most significant allele combinations that alter warfarin dosage are summarised in table 3.17 (section 3.2.2.5). The haplo.stats results suggest that some of the variants have larger effects on warfarin dosage than others and that the *VKORC1* variants may over-ride the effects of the *CYP2C9* variants. This hypothesis is supported by a recent study published in March 2008 by Schwarz et al., which showed that the initial variability in the INR response to warfarin was more strongly associated with genetic variability in *VKORC1* than with *CYP2C9*.

4.2.3 Pharmacogenomic testing in SA

Pharmacogenomic testing involves the observation of many genes or gene patterns and environmental factors that influence the action of a particular drug (Kalow, 2005). These tests are based on genetic markers that are observed at high frequencies within a particular population and whose influence on the drug of interest is known (The Royal Society, 2005). The genetic markers that are currently commonly used for pharmacogenomic testing of warfarin are *CYP2C9*2*, *CYP2C9*3* and *VKORC1* -1639 G>A (Hillman et al., 2005; Kimball Genetics Website). The frequency of the *VKORC1* -1639 G>A variant in

the SA black population is still unknown; however, neither of the *CYP2C9* variants are common in the SA black population (described in section 3.1). All three of these variants decrease warfarin dosage (the *VKORC1* variant is a promoter variant which switches off the gene thereby reducing warfarin dosage). Thus dosage is decreased according to the variants present in an individual.

In my study I observed 27 novel *CYP2C9* variants. Of the nine variants, observed at an allele frequency of 0.02 or above, five appear to influence warfarin dosage. Some of the remaining 18 variants may also influence warfarin dosage at a lower frequency in the SA black population, but remain to be assessed (discussed in section 4.1.3). Four of the five described *CYP2C9* and *VKORC1* variants (1, 2, 13 and 14) observed in the sample groups, were previously described as having no or a contradictory influence on warfarin dosage (*CYP2C9* Allele Nomenclature Database; D'Andrea et al., 2005), but appear to influence warfarin dosage in the patients in this. These results imply that none of the tests currently available to predict the most effective dosage of warfarin for a particular patient, thus reducing the risk of adverse effects, would be useful in the SA black population because of the differences in variant frequencies and contradictory effects. Warfarin is still the most frequently prescribed drug for the treatment and prevention of thromboembolic events. Thus a pharmacogenetic test, specific for this population, would need to be developed and implemented to ensure the safe administration of the drug to the vast number of patients requiring its treatment.

From this study I could develop a model aimed at screening patients for the common variants observed within the SA black population. This model could be used in a pilot study to determine the patients' initial warfarin dosage more effectively. The rare variants,

observed in the SA black population that could not be assessed in this study because the numbers were too small, may have importance in warfarin dosage. Thus, functional analysis could be carried out on all of the novel variants to determine their effect on warfarin metabolism. The frequencies of the *VKORC1* -1639 G>A promoter and 1173 C>T Intron 1 variants in the SA black population, not done in this study, would need to be determined. Based on these results the variants that appear to influence warfarin dosage could be included into the model used to determine warfarin dosage more effectively.

A study carried out by Wadelius et al. (2007) showed that variants within *CYP2C9*, *VKORC1*, *PROC*, *EPHX1*, *GGCX*, *ORM1* and *ORM2* with age, bodyweight and drug interactions account for approximately 73% of warfarin dosage variability. A larger study is currently ongoing in the United Kingdom (described in section 1.5) to assess the influence of a clinical, pharmacological, biochemical and haematological environmental factors and the 30 genes said to be involved in the mode of action of warfarin, on warfarin dosage variability (Wadelius et al., 2007). This study may identify the most significant environmental and genetic factors that contribute to warfarin dosage variability. The 14 *CYP2C9* and *VKORC1* variants and the small subset of environmental factors used to determine warfarin dosage variability in these patients only account for 45.3% of warfarin dosage variability. Thus I am currently unable to account for 54.7% of warfarin dosage variability in the SA black population.

Based on the results of this study, I would need to customise this test to the SA black population with the local variants; otherwise it would not provide useful information. I could also screen other SA populations such as Caucasian, Indian and mixed ancestry for the same factors and customise the tests according to the common variants within each

population. Once I have identified the most significant environmental factors and genetic variants at high frequencies within each SA population I could design a rapid screening test for these specific factors. This screening test would need to be verified and could eventually be offered as a pre-administration pharmacogenomic test for warfarin within the SA populations. This test would determine the initial warfarin dosage of a patient more efficiently, thereby decreasing the number of adverse effects the patient has to warfarin and shortening the time required to stabilise dosage.

4.3 Pregnancy Outcome Analysis

This section discusses the influence of a small subset of environmental factors and the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome in patients on warfarin during pregnancy. Section 4.3.1 discusses the influence of the environmental factors (age and concomitant medication) on pregnancy outcome. Section 4.3.2 discusses the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome. Section 4.2.3 discusses the implications of using these environmental factors and 14 *CYP2C9* and *VKORC1* variants as risk factors for poor pregnancy outcome in genetic counselling for pregnant mothers on warfarin.

4.3.1 The influence of the environmental factors on pregnancy outcome

4.3.1.1 Age and Heparin

Advanced maternal age typically refers to pregnant woman who will be ≥35 years of age on the estimated date of confinement (Fretts, 2007). The effects of increasing age occur as a continuum rather than a threshold effect (Fretts, 2007). When comparing the pregnancy outcome after the patients' first pregnancy with maternal age (shown in figure 3.16 in

section 3.3.1.1), I found that the women with more poor pregnancy outcomes had a median age of 29 and the women with more good pregnancy outcomes had a median age of 23.

Heparin taken during pregnancy is not teratogenic (Ginsberg et al., 2001). Consistent with this I observed that heparin decreased the risk of having a poor pregnancy outcome in the patients in both the linear and interaction models (tables 5.44-5.68 in Appendix L summarised in sections 3.3.2.2 and 3.3.2.3).

4.3.1.2 Warfarin

Warfarin is known to be teratogenic, resulting in a specific constellation of malformations known as fetal warfarin syndrome (FWS) (Hall et al., 1980), abnormal live borns and fetal loss (Cotrufo et al., 2002). In all of the linear and interaction models (Tables 5.44 – 5.68 in Appendix L, described in sections 3.3.2.2 and 3.3.2.3) I found that warfarin taken during pregnancy increased the risk of having a poor pregnancy outcome in the patients. I assessed the influence of warfarin dosage on pregnancy outcome by comparing the frequency of normal and poor pregnancy outcomes amongst patients on low, average and high warfarin doses (shown in figures 3.17 – 3.19 in section 3.3.1.3). These graphs show that there is an increase in poor pregnancy outcome with an increase in warfarin dosage. Pregnancy outcome with respect to warfarin dosage has not been previously studied in this manner.

A greater unbound fraction of warfarin has been found in the serum of pregnant women than non-pregnant women. It is this unbound fraction of warfarin that crosses the placenta and becomes teratogenic (Bajoria et al., 1996). The risk for pregnancy complications in patients treated with sodium warfarin is higher when the mean daily dose exceeds five mg (Cotrufo et al., 2002). I could hypothesise that patients on high doses of warfarin have a

greater fraction of unbound warfarin than those on low doses of warfarin. Thus more warfarin would cross the placenta and result in FWS. Warfarin dosage as a risk factor in poor pregnancy outcome in patients on warfarin will be discussed in section 4.3.3.

4.3.2 The influence of the 14 *CYP2C9* and *VKORC1* on pregnancy outcome

Table 3.23 (section 3.3.2.5) summarises the results of all of the analyses used to determine the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome in patients taking warfarin during pregnancy. Based on the warfarin dosage analysis I would expect that the variants that appear to increase warfarin dosage would increase the risk of having a poor pregnancy outcome. Table 4.6 summarises the influence of the 14 variants on warfarin dosage, what I expect and what I observed their influence on pregnancy outcome to be, based on their influence on warfarin dosage. In the table the blocks highlighted in orange are the variants that showed a decrease in warfarin dosage and decrease in the risk of having a poor pregnancy outcome. The blocks highlighted in yellow are the variants that showed an increase in warfarin dosage and an increase in the risk of having a poor pregnancy outcome.

Table 4.6: Summary of the influence of the 14 variants on warfarin dosage and their expected and observed influence on pregnancy outcome

Variant	Influence on warfarin dosage	Expected risk of poor pregnancy outcome	Observed risk of poor pregnancy outcome
1	Decreases	Decreased	Insignificant
2	Increases	Increased	Increased in one analysis
3	Decreases	Decreased	Decreased in one analysis
4	None	None	Insignificant
5	Increases	Increased	Insignificant
6	Decreases	Decreased	Insignificant
7	Decreases	Decreased	Insignificant
8	Decreases	Decreased	Insignificant
9	None	None	Decreased in one analysis
10	None	None	Decreased in one analysis
11	Decreases	Decreased	Decreased in one analysis
12	None	Contradictory	Insignificant
13	Increases	Increased	Decreased in two analyses
14	Increases	Increased	Insignificant

Results highlighted in orange = those that decrease warfarin dosage and decrease the risk of a poor pregnancy outcome. Results highlighted in yellow = those that increase warfarin dosage and increase the risk of a poor pregnancy outcome. Results that are not highlighted show no influence on warfarin dosage or pregnancy outcomes.

Most of the observed risks of having a poor pregnancy outcome (shown in table 4.6) were insignificant. Variant 2, however, was expected to increase the risk of having a poor pregnancy outcome and was observed to do so in one of the analyses I used. Similarly, variants 3 and 11 were expected to decrease the risk of having a poor pregnancy outcome and were observed to do so in one of the analyses I used, respectively. Variants 9 and 10 were expected to have no influence on pregnancy outcome, but showed a decrease in the risk of having a poor pregnancy outcome in one of the analyses. Variant 13, however, was expected to increase the risk of having a poor pregnancy outcome but decreased the risk in two of the analyses I used. There are no other studies comparing these variants and pregnancy outcome to my knowledge.

The numbers of patients with normal and poor pregnancy outcomes with the different variant genotypes were very small, which may account for the contradictory and insignificant results. Thus, further studies using a larger sample size of patients with pregnancies on warfarin may be carried out to verify our hypothesis that variants that result in an increase in warfarin dosage increase the risk of having a poor pregnancy outcome, due to the increase in warfarin dosage. In addition other factors that are known to cause poor pregnancy outcomes, such as additional drugs taken, the mother's diet and additional genetic factors, need to be taken into account when determining the influence of warfarin on pregnancy outcome.

4.3.3 Genetic counselling for pregnant women on warfarin

A new definition of genetic counselling has been approved by the National Society of Genetic Counselors (NSGC) (Resta et al., 2006). This definition states that genetic counselling is a process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease (Resta et al., 2006). Genetic counselling clinics are run at all the University of the Witwatersrand academic hospitals (Johannesburg General Hospital, Chris-Hani Baragwanath Hospital and Coronation Hospital) on a weekly basis. One of the many aims of these clinics is to counsel women who are taking warfarin and are pregnant or planning a pregnancy. These sessions aim to inform the patient about the risk of having a baby with fetal warfarin syndrome (FWS), the complications of warfarin during pregnancy, the critical period during which exposure to warfarin is said to result in FWS, the risks involved in taking warfarin past the critical period and the options that are available to the mother. All of the patients that attend these clinics are referred to the clinics by the doctors in those hospitals.

Despite its availability, Dr Gregersen's study (described in section 1.7) found that five (out of 124) patients from the Obstetric Cardiac Clinic at the CHB hospital, who were taking warfarin and were pregnant, received genetic counselling. These results suggest that these patients are not being referred to the clinics by their doctors. Thus information regarding the availability and importance of these clinics needs to be drastically improved. Women on child-bearing age (wanting to have children), on warfarin treatment would need to be seen and counselled pre-pregnancy to assess whether their pregnancy can be managed off warfarin. If the pregnancy can not be managed off warfarin they would need to be counselled concerning the risks to themselves and their baby, refer to table 4.6.

From this study I have identified four variants (2 *CYP2C9* (variants 2 and 5) and two *VKORC1* (variants 13 and 14)) variants that could increase the risk of having a poor pregnancy outcome, through an increase in warfarin dosage; and six *CYP2C9* variants (variants 1, 3, 6, 7, 8 and 11) that could decrease the risk of having a poor pregnancy outcome, through a decrease in warfarin dosage (shown in table 4.6). These results would need to be verified using larger sample sizes. Assuming that these hypotheses are correct I would need to develop a rapid method to screen patients for these variants. This test could be used to identify patients at a higher risk of having a poor pregnancy outcome, altering their dosage accordingly and thereby reducing the risk of complications to both the mother and baby.

4.4 Limitations

In this study I had small sample numbers, limiting the types and number of analyses I could do on the different variants. I only assessed two (*CYP2C9* and *VKORC1*) of the possible 30 genes said to be involved in the action of warfarin as they are the most

important of all the genes that interact with warfarin. However, the 28 remaining genes may play an important role in warfarin dosage variability in the SA populations. Thus further studies aimed at determining the frequencies of variant alleles for these 28 genes and their effect on warfarin dosage in the SA populations should be carried out.

In terms of designing a broad scale pharmacogenomic project, aimed at providing a safer, more efficient way of administering warfarin in SA, one could say our limitations would be the fact that pharmacogenetics, let alone pharmacogenomics is not yet adequately established in SA and we do not have the funding or technology to carry out such a study. In addition clinicians might say that the cost of requiring a pre-prescription test for warfarin (or any other drug) may outweigh the benefit. This may be so for now but we are seeing an ongoing trend of making technology and genetic testing more cost effective. It has been my experience over the past three years, this year in particular, that scientists and clinicians tend to use these “limitations” as excuses to either run away from the country or continue with the small scale science that we tend to produce. We think that because we come from a developing country that we are unable to produce internationally competitive science, which is really sad considering the level of expertise we have in this country. How then do we change this mindset or start to achieve the type of science and launch projects that reflect this expertise? It was highlighted to me by three international speakers, at a recent conference I attended this year, that we as South Africans tend to keep our work to ourselves and not collaborate with other countries especially first world countries who have the resources that we complain we do not have. Of course this is a broad generalisation which is not to say that we do not collaborate at all but we certainly do not do it enough. Why then do we not plan bigger more competitive projects with the help of collaborators that have the resources we need, until we are able to obtain these resources

ourselves, and even then share intellectual properties? I believe that genomic studies will overshadow genetic studies and that they will become more cost effective. Our job at this point is making sure we are ready for this transition.

5 CONCLUSION

In this study I determined the frequencies of previously described and novel *CYP2C9* and *VKORC1* variants and how these variants influence warfarin dosage variability and poor pregnancy outcome (when warfarin is taken during pregnancy), in the South African (SA) black population. I observed six of the 30 previously described *CYP2C9* variants, 27 novel *CYP2C9* variants and three of the 11 previously described *VKORC1* variants, but no novel *VKORC1* variants in the patient and control samples. Of the 27 novel *CYP2C9* variants, four are missense mutations, four are silent mutations, nine are possible splice site mutations and ten are of unknown effect. Of the 36 *CYP2C9* and *VKORC1* variants observed in the patient and control samples, only 14 were observed at an allele frequency of ≥ 0.02 and were used for further analyses. Of these 14 variants, three were previously described *CYP2C9* variants, nine novel *CYP2C9* variants and two previously described *VKORC1* variants. When comparing the allele and genotype frequencies of the patient samples to the control samples I found no significant difference between the two. Thus the results obtained through the warfarin dosage variability and pregnancy outcome analyses (using only the patient samples) apply to the general SA black population.

In the warfarin dosage variability analysis I found that beta blockers were the only environmental factors that significantly influenced warfarin dosage across all of the analyses. Beta blockers appear to decrease warfarin dosage by eight mg/week (on average). These results imply that one could reduce the risk of haemorrhagic complications in patients taking both warfarin and beta blockers by decreasing their warfarin dosage by approximately eight mg/week.

I observed six of the 30 previously described *CYP2C9* variants, one of which (*CYP2C9*11*) is known to decrease warfarin dosage through a decrease in warfarin metabolism (*CYP2C9* Allele Nomenclature Database). *CYP2C9*9*'s influence on warfarin dosage has been previously unknown (*CYP2C9* Allele Nomenclature Database). *CYP2C9*8*'s influence on warfarin dosage was described as contradictory (*CYP2C9* Allele Nomenclature Database). In the patients *CYP2C9*8* and *CYP2C9*11* appear to decrease warfarin dosage and *CYP2C9*9* appears to increase warfarin dosage by approximately 12, four and nine mg/week, respectively.

I observed 27 novel variants within the *CYP2C9* gene, nine of which were observed at a frequency of ≥ 0.02 and were used for further analysis. Of these 9, one (variant 5 – possible splice site mutation) appears to increase warfarin dosage, four (variants 6 – unknown mutation, seven – possible splice site mutation, eight – possible splice site mutation and 11 – silent mutation) appear to decrease warfarin dosage, while the remaining four appear to have no or a contradictory influence on warfarin dosage.

I observed three of the 11 previously described *VKORC1* variants. The L120L *VKORC1* variant was described as having no influence on warfarin dosage (D'Andrea et al., 2005); however, it appears to increase warfarin dosage in the patients in this study by five mg/week. The 3730 G>A *VKORC1* variant's influence on warfarin dosage was unknown (D'Andrea et al., 2005), but appears to increase warfarin dosage in the patients in this study by four mg/week. The V66M *VKORC1* variant was observed at an allele frequency of 0.01 within the patients and was therefore not used in any warfarin dosage or pregnancy outcome analyses.

All of the environmental factors (age and concomitant medications) and the 14 *CYP2C9* and *VKORC1* variants used in the warfarin dosage variability analyses in this study account for 45.3% of warfarin dosage variability in the SA black population. The 14 *CYP2C9* and *VKORC1* variants alone account for 34.7% of warfarin dosage variability in the SA black population, and are thus significant contributions to warfarin dosage variability.

Warfarin has teratogenic effects (Hall et al., 1980; Cotrufo et al., 2002). In the pregnancy outcome analysis I found that heparin, consistent with previous studies, taken during pregnancy significantly decreases the risk of having a poor pregnancy outcome, compared to warfarin throughout pregnancy. An increase in maternal age and warfarin both significantly increase the risk of having a poor pregnancy outcome. When assessing pregnancy outcome with respect to warfarin dosage I found that there was an increase in the number of poor pregnancy outcomes with an increase in warfarin dosage. I hypothesised that patients taking a high dose of warfarin have a higher fraction of unbound warfarin which then crosses the placenta, exposing the developing fetus to higher doses of warfarin which then results in fetal warfarin effects. Thus patients taking a high dose of warfarin are at a higher risk of having a poor pregnancy outcome than those on a lower dose of warfarin.

Based on the warfarin dosage vs. pregnancy outcome analysis I hypothesised that the variants that result in an increase in warfarin dosage would increase the risk of having a poor pregnancy outcome; and that variants that result in a decrease in warfarin dosage would decrease the risk of having a poor pregnancy outcome. When analysing the 14 *CYP2C9* and *VKORC1* variants I found that the *CYP2C9*9* variant that increases warfarin

dosage, increased the risk of having a poor pregnancy outcome. *CYP2C9*11* and a novel *CYP2C9* variant (11) which decrease warfarin dosage, decreased the risk of having a poor pregnancy outcome. Two variants that appear to have no influence on warfarin dosage (variants 9 and 10) appear to decrease the risk of having a poor pregnancy outcome. The L120L *VKORC1* variant (variant 13) which appears to increase warfarin dosage decreases the risk of having a poor pregnancy outcome. The influence of these variants on pregnancy outcome would need to be studied further, using a larger sample size.

Future studies arising as a result of this study involve:

- Development of a model, which screens for the most common *CYP2C9* and *VKORC1* variants in the SA black population, and the use of this model in a pilot study to determine warfarin dose more effectively.
- Functional analysis of all 27 novel *CYP2C9* variants.
- Determining the frequencies of the *VKORC1* -1639 G>A and 1173 C>T variants in the SA black population.
- Screen for variants in the promoter and regulatory regions, which could influence gene expression.
- Screening for genetic variants in the remaining 28 genes (or the most significant) and identifying the most important environmental factors said to be involved in the mode of action of warfarin (described in section 1.5) in all SA populations (Black, Caucasian, Asian and mixed ancestry).
- Development of a rapid detection method to screen for the most frequent genetic (other than the 14 *CYP2C9* and *VKORC1* variants identified in this study) variants whose influence on warfarin dosage is known in SA populations to predict warfarin dosage more effectively.

- Verifying this test for the use of pre-administration pharmacogenomic testing for warfarin in SA populations.
- Studying the influence of the different *CYP2C9* and *VKORC1* variants observed in the SA black population on pregnancy outcome in a larger sample size.
- Setting up a rapid screening test to identify patients at an increased risk of having a poor pregnancy outcome.

From this project I was able to provide more informative genetic counselling for patients on warfarin who are having or plan to have children, based on the knowledge of warfarin dosage and pregnancy outcomes. In addition this project has provided vital information into which *CYP2C9* and *VKORC1* variants are most common in the SA black population. Fourteen of these variants account for 34.7% of warfarin dosage variability in the SA black population.

In November 2005, the Clinical Pharmacology Subcommittee (in the USA) agreed that sufficient evidence exists to support use of lower doses of warfarin for patients with genetic variants in *CYP2C9* and *VKORC1* that lead to reduced activities (based on the *CYP2C9*2*, *CYP2C9*3* and *VKORC1* -1639 G>A variants) (Kimball Genetics Website). A label change for warfarin is underway to reflect this recommendation (Kimball Genetics Website). None of the current pre-administration pharmacogenetics/genomics tests for warfarin are useful in the SA black population. It is thus imperative that a pre-administration pharmacogenetics test for warfarin, specifically for SA black patients, be established. The accomplishment of this has been made easier through the information obtained in this project. It is my hope that in the near future similar studies will be established for all SA populations, providing the necessary information for the

development of pre-administration pharmacogenetics tests for warfarin for all SA populations, and perhaps the eventual establishment of larger pre-administration pharmacogenomics tests for warfarin for all SA populations.

Appendix A

Solutions:

3% Agarose Gel

12g Agarose powder

400ml 1 x TBE

12µl EtBr (10mg/ml)

Dissolve agarose and TBE in microwave

Once cooled add EtBr

Pour gel into a gel tray with combs to form wells and allow setting for approximately 30min –one hour.

0.5M EDTA

93.06g EDTA in 400ml ddH_2O

pH to 8.0 with NaOH pellets

Make up to 500ml

Autoclave

70% Ethanol

70ml 100% Ethanol + 30ml ddH_2O

Ethidium Bromide (EtBr) (10mg/ml)

10mg EtBr powder

1ml ddH_2O

Store in a dark bottle

Also commercially available from Sigma as an aqueous solution (10mg/ml)

Ficoll-bromophenol blue loading dye

50g sucrose crystals

0.1ml 0.5M EDTA (pH 7.0)

0.1g Bromophenol blue dye

10g Ficoll powder

Make up to 100ml with ddH_2O

Aliquot into 1.5ml Eppendorf tubes

Store at 4˚C

1M $MgCl_2$

50.83g $MgCl_2$

250ml ddH_2O

Autoclave

1kb+ Molecular weight DNA marker

11µl ladder

10µl Ficoll loading dye

73µl 1x TE

Proteinase K solution (16 extractions)

800µl 10% SDS

32µl 0.5M EDTA

5.6ml autoclaved ddH_2O

Add 1600µl 10mg Proteinase K just before use

Saturated NaCl

100ml autoclaved ddH_2O

Slowly add 40g NaCl until absolutely saturated

Before use, agitate and let NaCl precipitate out

10% SDS

10g/100ml ddH_2O

Sucrose-Triton X Lysing Buffer

10ml 1M Tris-HCl (pH8)

5ml 1M $MgCl_2$

10ml Triton-X 100

Make up to 1l with ddH_2O

Autoclave

Add 109.5g Sucrose just before use

Keep solution chilled at 4˚C

T20E5

20ml 1M Tris-HCl (pH8)

10ml 0.5M EDTA (pH8)

Top up to 1l volume with ddH_2O

Autoclave

10 x Tris Borate EDTA (TBE)

109.02g Tris base

55.64g Boric acid

7.44g NaEDTA

Adjust pH to 8.3 with HCl

Make up to 1l with dH_2O

Dilute 10-fold before use

Store at room temperature

1 x Tris Borate EDTA (TBE)

Dissolve 100ml 10 x TBE in 900ml dH_2O

1 x Tris-EDTA (TE) Buffer

10 ml 1M Tris-HCl (pH8.0)

2 ml 0.5M EDTA

Make up to 1l with dH_2O

Autoclave and store at room temperature.

1M Tris-HCl (pH 8)

121.1g Tris in 1l ddH_2O

pH to 8.0 using concentrated HCl

Make up to 1l

Autoclave

Appendix B

Salting-out DNA Extraction Procedure (Miller et al., 1988)

- Collect blood into a ACD or EDTA tube
- Decant no more than 10ml of blood into a Nunc tube
- Spin for ten minutes to remove plasma and freeze at -20˚C until extraction or begin immediately
- Fill each tube to the 45/50ml mark with chilled Sucrose-Triton X Lysing Buffer
- Invert the tube to mix
- Spin for ten minutes at 2300rpm
- Pour off the supernatant (the pellet should be reddish)
- Resuspend the pellet in 20-25ml Sucrose-Triton X Lysing Buffer and place on ice for five minutes
- Spin for five minutes at 2300rpm
- Pour off the supernatant carefully (the pellet should now be pinkish/white)
- Add the following to the pellet:
  - 3ml T20E5
  - 200µl 10% SDS
  - 500µl Proteinase K solution

  Mix by inversion
- Incubate the samples at 42˚C to 50˚C overnight
- Add 1ml of saturated NaCl and mix vigorously for 15 seconds
- Spin for 15 minutes at 2500rpm
- A white pellet should be visible which consists of protein and precipitated salt
- Transfer the supernatant that contains the DNA to a new Nunc tube
- Add two volumes alcohol to the supernatant
- Agitate gently and spool DNA
- Wash in 70% ice-cold ethanol
- Place DNA in an eppendorf tube and air dry
- Dissolve the DNA in the appropriate volume of TE buffer (200µl - 1000µl)

Appendix C

Table 5.1: Primer sets for both *CYP2C9* and *VKORC1* gene amplicons

CYP2C9		*VKORC1*	
Amplicon	**Primer**	**Amplicon**	**Primer**
Exon 1	F: 5'-ttt ttt att acc aat acc tag g-3'	Exon 1	F: 5'-tcg ctg ttt tcc taa ctc-3'
	R: 5'-ttt tac ttt acc att acc tct tg-3'		R: 5'-ccg atc cca gac tcc aga at-3'
Exons 2 & 3	F: 5'-tac aaa tac aat gaa aat atc atg-3'	Exon 2	F: 5'-tga cat gga atc ctg acg tg-3'
	R: 5'-cta aca acc agg act cat aat g-3'		R: 5'-gag ctg acc aag ggg gat-3'
Exon 4	F: 5'-tgt taa ggg aat ttg tag g-3'	Exon 3	F: 5'-agt gcc tga agc cca cac-3'
	R: 5'-aat ttt gga ttt gtc aga a-3'		R: 5'-ata acc acc ctt ccc agc ag-3'
Exon 5	F: 5'-cag agc ttg gta tat ggt atg-3'		
	R: 5'-gta aac aca gaa cta gtc aac-3'		
Exon 6	F: 5'-gtt tgg gca agt tgg tct a-3'		
	R: 5'-aga aac agg aag gag gac ac-3'		
Exon 7	F: 5'-ctc ctt ttc cat cag ttt tta ct-3'		
	R: 5'-gat act atg aat ttg ggg act tc-3'		
Exon 8	F: 5'-ttc atg gct tct tta cag ct-3'		
	R: 5'-tcc cca aag tcc act aat ct-3'		
Exon 9	F: 5'-tat tgc ata ttc tgt ttg tgc-3'		
	R: 5'-caa gta act cta aca ctc acc c-3'		

Table 5.2: 10x dNTP Mix

DNTP Component	[Stock]	[Final]	1ml mix
dATP	10mM	1.25mM	125µl
dCTP	10mM	1.25mM	125µl
dTTP	10mM	1.25mM	125µl
dGTP	10mM	1.25mM	125µl
ddH_2O			500µl

Table 5.3: PCR Mix

PCR Mix	[Stock]	[Final]	1 x Mix
DNA			1 µl
dNTP's	10 x	1 x	2.5 µl
Buffer	10 x	1 x	2.5 µl
$MgCl_2$	10 x	1 x	2.5 µl
Primer F	100 ρM	10 ρM	1 µl
Primer R	100 ρM	10 ρM	1 µl
Ampli Taq	5U/µl	1U/µl	0.2 µl
dH_2O			14.3 µl
Total Volume			**25 µl**

Table 5.4: PCR Conditions for all *CYP2C9* and *VKORC1* amplicons

Initial Denaturation	Denaturation	Annealing (40 seconds)		Extension	Final Extension	Hold
95˚C for 5 minutes	**30 Cycles**				72˚C for 7 minutes	15˚C ∞
	95˚C for 40 seconds	***CYP2C9***	Ex 1 – 51˚C	72˚C for 1 minute 20 seconds		
			Ex 2&3 - 55˚C			
			Ex 4 - 51˚C			
			Ex 5 - 51˚C			
			Ex 6 - 60˚C			
			Ex 7 - 60˚C			
			Ex 8 - 52˚C			
			Ex 9 - 59˚C			
		VKORC1	Ex 1 - 55˚C			
			Ex 2 - 61˚C			
			Ex 3 - 64˚C			

Appendix D

Table 5.5: *Ava*II Digest for *CYP2C9*2*

Digest Mix	[Stock]	[Final]	1 x Mix
PCR Product			20µl
*Ava*II	10U/µl	2.5U/µl	0.25µl
Buffer R*	10x	1x	2.5µl
ddH_2O			2.25µl
Total Volume			**25µl**

* Provided with the enzyme.

Digest for 2hrs at 37ºC
Run products on 3% Agarose Gel

Table 5.6: *Alu*II Digest for *CYP2C9*5*

Digest Mix	[Stock]	[Final]	1 x Mix
PCR Product			20µl
*Alu*I	10U/µl	2.5U/µl	0.25µl
Buffer R*	10x	1x	2.5µl
ddH_2O			2.25µl
Total Volume			**25µl**

* Provided with the enzyme.

Digest for 2hrs at 37ºC
Run products on 3% Agarose Gel

Appendix E

Multiscreen ® $PCR_{\mu 96}$ Cleanup Protocol

- Place 20µl (remaining volume after 5µl was used to check for product on the 3% agarose gel) of PCR mix into the wells of the Multiscreen ® $PCR_{\mu 96}$ Cleanup Filter plate.
- Place the plate on the vacuum filtration manifold (Millipore Millivac ® Maxi SD1P014M04) and vacuum for approximately 2-3 minutes or until the beds are dry at 260kPa (the maximum pressure the vacuum could obtain at a high altitude (Johannesburg – approximately 2000m)).
- Add 20µl of ddH_2O to each of the wells.
- Place the plate on a shaker (Labnet Orbit 1000) for two minutes at 300rpm.
- Resuspend the samples by pipetting up and down approximately four times.
- Transfer the samples into a new 96-well PCR plate, cover and store at 4ºC until needed.

Appendix F

Table 5.7: Sequencing Reaction Setup (1/8x)

Final Reaction Volume:	5µl	10µl
PCR (10-50fmol/well)	2µl	2µl
AB1 5x Sequencing Buffer	0.5µl	1.5µl
Primer (5 pmol/µl)	1µl	1µl
BDT Premix	1µl	1µl
Milli-Q Water	0.5µl	4.5µl

Appendix G

Montage SEQ_{96} Sequencing Reaction Cleanup Kit Protocol:

- Dilute samples with 20µl of the injection solution (provided in the Montage Kit).
- Mix by pipetting approximately four times.
- Transferred the samples to the SEQ_{96} cleanup plate and place the plate onto the vacuum manifold.
- Vacuum at approximately 260kPa for 2-3 minutes or until the membrane is dry.
- Remove the plate from the vacuum and blot of excess liquid.
- Add a further 30µl of injection solution to each well and replaced the plate on the vacuum manifold.
- Vacuum for approximately 3-4 minutes or until the membrane is dry.
- Re-blot to remove excess liquid.
- Add a final volume of 20µl injection fluid to each well.
- Place the plate on a shaker (Labnet Orbit 1000) and shake at 300 rpm for approximately two minutes.
- Resuspend samples by pipetting up and down approximately four times.
- Transfer the samples to a new 96-well plate with an injection cover for sequencing.

Appendix H

Protocol on how to create and start a Sequencing run

Plate Design

- On the desktop of the computer in which the 3130xl Genetic Analyser's software is loaded, click on the plate manager application
- Click on new plate. A plate dialogue will open up.
- In the plate dialogue you describe the name of the plate, the type of application (sequencing or microsatellite detection), type of plate (96 or 384 well), the owner and operator's names. Click ok and a sequencing plate editor application will open.
- In the sequencing plate editor application you describe each sample's name, results group, and instrument and analysis protocols.
- The results group is a folder in which all results will be stored.
- The instrument protocol refers to the type of polymer, the capillary length and programme with which the samples will be run. The two protocols I used are Z.SEQ_Pop7.36.Rapid and Z.SEQ_Pop7.36.Ultra. These protocols used polymer seven (pop7) with a 36cm array. The rapid protocol is used for fragments above 500bp in length and the ultra is used for fragments of below 500bp in length.
- The analysis protocol refers to the type of analysis. I used 3130 Pop7_BDTv3kb (3130 polymer 7, Big Dye Terminator version 3).

Starting the Sequencing run

- Click on the Run scheduler application.
- Click on plate view and update the list by clicking on "find all".
- Find the name of your plate (specified in the plate dialogue).
- Click on the name of the plate and then the position of the plate (A or B), to link the name of your plate to the position of the plate in the genetic analyser.
- Go to the run view, check that you have linked the correct plate and runs.
- Click on the play button to start the run and confirm that you want to start the run by clicking "ok".

Appendix I

Protocol to Retrieve and Analyse a Sequencing run

- Open the Sequencing Analysis 5.2 software by clicking on the short cut icon
- Click on “file” and go to add samples
- Find the name of the results group (specified in the sequencing plate editor)
- Highlight the samples you wish to add and click on add selected samples and then ok
- Click on the play button and the software will automatically analyse, allocate and call a base to each peak.
- Each sample may be viewed as an electropherogram by clicking on the name of the sample.
- The view of the electropherogram may be adjusted by making the peaks wider or closer together, higher or smaller by clicking on the appropriate icons.
- When you close the application you have the option of saving the samples, which will then be stored in the results group folder and may then be transferred to a flash disk or printed using a laser printer.

Appendix J

Statistical Values for the Variant Analyses

All p-values highlighted in the tables are smaller than 0.05 and were considered significant.

Table 5.8: Exact test for Hardy-Weinberg Equilibrium for all sample groups

Variant	P-Values		
	Controls	Patients	Both
1	1	1	1
2	0.1416	0.518	1
3	0.1714	1	0.2827
4	0.1731	1	0.5269
5	0.4547	1	0.7782
6	0.6858	0.3709	0.7743
7	0.6116	0.3709	0.1942
8	0.2654	0.1071	0.5945
9	1	1	1
10	1	1	0.6067
11	1	1	0.6052
12	1	0.3709	0.7114
13	0.7572	0.8058	1
14	0.3043	0.702	0.2644

Table 5.9: P-values of Linkage Disequilibrium for Control Samples

Variant	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.1063	0.7145	0.9946	0.9955	0.9953	0.9941	0.8998	0.9626	0.2611	0.2611	0.7904	0.0863	0.0322
2		0.0762	0.9968	0.9974	0.4244	0.1494	0.9974	0.9751	0.995	0.995	0.08	0.0876	0.4268
3			0.9919	0.6789	0.2709	0.1416	0.1103	0.9336	0.9855	0.9855	0.0667	0.0476	0.3978
4				0.9995	0.9848	0.3732	0.9982	0.9761	0.9952	0.9952	0.9943	0.1384	0.1102
5					0.9967	0.3955	0.6494	0.9801	0.996	0.996	0.9953	0.3663	0.1149
6						0.6733	0.6872	0.9794	0.9958	0.7126	0.9951	0.032	0.0056
7							0.2911	0.027	0.9947	0.9775	0.9938	0.1755	0.1811
8								0.987	0.9974	0.7721	0.9063	0.3745	0.0247
9									0.9573	0.9573	0.9648	0.0659	0.4526
10										0.766	0.9929	0.3725	0.5225
11											0.9929	0.3488	0.4848
12												0.0219	0.0504
13													0.9996

1 = Linkage disequilibrium (LD), 0 = no LD. Significant values seen between variants 1-12 and 13-14 are not a measure of LD but rather show the presences of the same variants between individuals. The values in the table show the D' measure.

Table 5.10: P-values of Linkage Disequilibrium for Patient Samples

Variant	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.0184	0.9855	0.995	0.9971	0.5789	0.1717	0.7759	0.9795	0.9911	0.9911	0.9161	0.1942	0.1389
2		0.2362	0.9979	0.9981	0.998	0.998	0.423	0.7703	0.0058	0.169	0.0889	0.3992	0.4209
3			0.9839	0.986	0.003	0.9845	0.4906	0.923	0.969	0.9711	0.9845	0.5138	0.3316
4				0.9995	0.9968	0.9966	0.9986	0.3471	0.9901	0.9901	0.9958	0.4756	0.4243
5					0.6135	0.3628	0.8193	0.3166	0.9914	0.9914	0.9956	0.6454	0.2658
6						0.8344	0.9429	0.978	0.9904	0.9904	0.9964	0.1566	0.2215
7							0.831	0.978	0.9904	0.9904	0.2291	0.2519	0.0791
8								0.9905	0.9959	0.9959	0.757	0.1703	0.0879
9									0.956	0.9589	0.978	0.9894	0.9938
10										0.9987	0.9904	0.2607	0.5446
11											0.9904	0.1967	0.3742
12												0.0459	0.0273
13													0.9995

1 = Linkage disequilibrium (LD), 0 = no LD. Significant values seen between variants 1-12 and 13-14 are not a measure of LD but rather show the presences of the same variants between individuals. The values in the table show the D' measure.

Table 5.11: P-values of Linkage Disequilibrium for both Control and Patient Samples

Variant	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.0671	0.9822	0.994	0.9949	0.996	0.9939	0.8223	0.9735	0.8538	0.9197	0.8479	0.0998	0.1102
2		0.0542	0.9959	0.9964	0.7097	0.6248	0.4771	0.981	0.9933	0.8628	0.0961	0.1143	0.881
3			0.9843	0.9866	0.1564	0.0428	0.012	0.9284	0.9708	0.9718	0.3928	0.2834	0.0184
4				0.9995	0.9958	0.8072	0.9985	0.0083	0.9903	0.9903	0.994	0.3044	0.2674
5					0.7255	0.3738	0.7622	0.3149	0.9917	0.9917	0.9949	0.5059	0.1922
6						0.765	0.8078	0.9786	0.9911	0.9911	0.995	0.0834	0.1181
7							0.5999	0.9761	0.9901	0.9901	0.6419	0.2158	0.0108
8								0.989	0.9954	0.9954	0.8171	0.1903	0.0503
9									0.9565	0.958	0.9729	0.2265	0.1852
10										0.8839	0.9888	0.3165	0.5316
11											0.9888	0.2671	0.4362
12												0.0106	0.0373
13													0.9995

1 = Linkage disequilibrium (LD), 0 = no LD. Significant values seen between variants 1-12 and 13-14 are not a measure of LD but rather show the presences of the same variants between individuals. The values in the table show the D' measure.

Table 5.12: P-values from the Fisher's Exact and Cochran/Armitage tests

Variant	P-values	
	Fisher's Exact Test	**Cochran/Armitage Test**
1 - *CYP2C9**8 R150H	0.1714	0.07717
2 - *CYP2C9**9 H251R	0.06649	0.1024
3 - *CYP2C9**11 R355W	0.8884	0.6278
4 - *CYP2C9* 12930T>C	0.4858	0.8646
5 - *CYP2C9* 16090 T>C	0.8764	0.7099
6 - *CYP2C9* 16179 T>A	0.3199	0.5256
7 - *CYP2C9* 21711 G>C	1	0.7871
8 - *CYP2C9* 46028 A>G	0.05715	0.1854
9 - *CYP2C9* 46092 C>T	1	0.8604
10 - *CYP2C9* 60272 T>C	0.8396	0.729
11 - *CYP2C9* A441A	1	0.8778
12 - *CYP2C9* 63113 C>T	0.307	0.2003
13 - *VKORC1* L120L	0.2798	0.1449
14 - *VKORC1* 3730 G>A	0.8238	0.8615

Fisher's exact test = based on genotype frequencies; Cochran/Armitage Test = based on allele frequencies

Appendix K

Statistical Values for the Dosage Analyses

All p-values highlighted in the tables are smaller than 0.05 and were considered significant.

Table 5.13: P-values of the influence of the concomitant drugs on warfarin dosage based on the Wilcoxon test

Drug	P-value
Digoxin	0.7587
Lasix	0.115
Slow K	0.1409
Beta Blockers	0.002019
Aspirin	0.06759
Aldactone	0.5793
Moduretics	0.8573
Ace Inhibitors	0.5502

Table 5.14: P-values indicating the influence of the 14 *CYP2C9* and *VKORC1* variants based on the Kruskal-Wallis test

Variants	Chi-squared	Degrees Freedom	P-value
1 - *CYP2C9**8 R150H	7.6623	2	0.02168
2 - *CYP2C9**9 H251R	3.8516	2	0.1458
3 - *CYP2C9**11 R355W	0.6769	1	0.4106
4 - *CYP2C9* 12930T>C	1.5347	2	0.4642
5 - *CYP2C9* 16090 T>C	2.3641	2	0.3066
6 - *CYP2C9* 16179 T>A	5.6741	2	0.0586
7 - *CYP2C9* 21711 G>C	1.8214	2	0.4022
8 - *CYP2C9* 46028 A>G	6.859	2	0.0324
9 - *CYP2C9* 46092 C>T	0.2129	1	0.6445
10 - *CYP2C9* 60272 T>C	0.3994	1	0.5274
11 - *CYP2C9* A441A	0.6458	1	0.4216
12 - *CYP2C9* 63113 C>T	5.2319	2	0.0731
13 - *VKORC1* L120L	4.9481	2	0.08424
14 - *VKORC1* 3730 G>A	2.1104	2	0.3481

Warfarin Dosage Linear Models

Tables 5.15 – 5.33 represent all of the linear models that were created to assess the impact of all of our environmental factors and 14 *CYP2C9* and *VKORC1* variants on warfarin dosage. In each table the intercept represents the warfarin dosage, in mg/week, of a patient who is not taking any concomitant medication, is 0 years old and has the wildtype allele for all 14 variants. The percentage variability (described in section 3.2.2.4) was determined by multiplying the multiple r-squared values by 100. The estimated standard (described in section 3.2.2.4) shows the influence of each coefficient on warfarin dosage as compared to the intercept dosage. In each model N/V represents the heterozygous genotype and V/V represents the homozygous variant genotype.

Table 5.15: Results of the linear model showing the influence of the four concomitant medications on warfarin dosage

Coefficients	Estimated Standard [3]	P-value
Intercept [1]	43.26	<2e-16
Lasix	-3.075	0.5590
Slow K	0.661	0.9020
Beta Blockers	-7.261	0.0310
Aspirin	3.904	0.1590
Model p-value	0.028	
Multiple r-squared [2]	0.0975	9.7%

[1]Intercept = warfarin dosage in mg/week if the patient is not taking any of the concomitant medications. [2] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [3] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.16: Results of the linear model showing the influence pact of the four concomitant medications and age on warfarin dosage

Coefficients	Estimated Standard [3]	P-value
Intercept [1]	51.2414	4.44E-09
Lasix	-3.1892	0.5448
Slow K	1.3030	0.8088
Beta Blockers	-7.3299	0.0292
Aspirin	3.6735	0.1858
Age	-0.2324	0.3012
Model p-value	0.03606	
Multiple r-squared [2]	0.1068	10.6%

[1] Intercept = warfarin dosage in mg/week if the patient is 0 years old and is not taking any of the concomitant medications. [2] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [3] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.17: Results of the linear model showing the influence of variant 1 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	51.677	2.1E-09
N/V [1]	-8.076	0.0092
V/V [2]	-1.063	0.91
Lasix	-4.085	0.4285
Slow K	3.061	0.564
Beta Blockers	-7.578	0.0215
Aspirin	3.71	0.1718
Age	-0.203	0.3607
Model p-value	0.00887	
Multiple r-squared [4]	0.165	16.5%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.18: Results of the linear model showing the influence of variant 2 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	52.161	2.4E-09
N/V [1]	0.449	0.875
V/V [2]	18.396	0.063
Lasix	-2.476	0.639
Slow K	1.45	0.788
Beta Blockers	-6.819	0.044
Aspirin	4.456	0.118
Age	-0.295	0.194
Model p-value	0.0315	
Multiple r-squared [4]	0.137	13.7%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.19: Results of the linear model showing the influence of variant 3 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [4]	P-value
Intercept [2]	52.58	2.8E-09
N/V [1]	-5.619	0.257
Lasix	-2.796	0.596
Slow K	0.743	0.891
Beta Blockers	-7.385	0.028
Aspirin	3.846	0.166
Age	-0.259	0.252
Model p-value	0.0405	
Multiple r-squared [3]	0.118	11.8%

[1] N/V = heterozygous genotype. [2] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [3] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [4] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.20: Results of the linear model showing the influence of variant four genotypes on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	51.01	1.1E-08
N/V [1]	3.071	0.322
V/V [2]	-4.273	0.756
Lasix	-4.206	0.434
Slow K	2.113	0.699
Beta Blockers	-7.507	0.026
Aspirin	3.807	0.174
Age	-0.241	0.294
Model p-value	0.0738	
Multiple r-squared [4]	0.116	11.6%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.21: Results of the linear model showing the influence of variant 5 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	51.77	9.E-09
N/V [1]	3.74	0.214
V/V [2]	-8.64	0.387
Lasix	-4.24	0.425
Slow K	2.73	0.617
Beta Blockers	-7.57	0.024
Aspirin	4.47	0.114
Age	-0.28	0.228
Model p-value	0.0466	
Multiple r-squared [4]	0.128	12.8%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.22: Results of the linear model showing the influence of variant 6 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	55.158	5.6E-10
N/V [1]	-8.474	0.0084
V/V [2]	13.425	0.1513
Lasix	-3.746	0.4614
Slow K	0.88	0.8653
Beta Blockers	-8.733	0.008
Aspirin	3.385	0.2064
Age	-0.279	0.2081
Model p-value	0.00304	
Multiple r-squared [4]	0.186	18.6%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.23: Results of the linear model showing the influence of variant 7 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	50.911	6.3E-09
N/V [1]	-4.403	0.169
V/V [2]	14.531	0.13
Lasix	-2.671	0.608
Slow K	0.175	0.974
Beta Blockers	-8.04	0.016
Aspirin	3.502	0.202
Age	-0.192	0.39
Model p-value	0.0221	
Multiple r-squared [4]	0.145	14.5%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.24: Results of the linear model showing the influence of variant 8 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	55.323	1.1E-09
N/V [1]	-3.927	0.161
V/V [2]	-8.794	0.037
Lasix	-3.897	0.454
Slow K	1.868	0.725
Beta Blockers	-7.725	0.021
Aspirin	3.367	0.221
Age	-0.276	0.222
Model p-value	0.0164	
Multiple r-squared [4]	0.151	15.1%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.25: Results of the linear model showing the influence of variant 9 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [4]	P-value
Intercept [2]	51.249	5.4E-09
N/V [1]	-0.165	0.981
Lasix	-3.188	0.547
Slow K	1.318	0.809
Beta Blockers	-7.343	0.032
Aspirin	3.672	0.188
Age	-0.233	0.303
Model p-value	0.0654	
Multiple r-squared [3]	0.107	10.7%

[1] N/V = heterozygous genotype. [2] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [3] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [4] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.26: Results of the linear model showing the influence of variant 10 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [4]	P-value
Intercept [2]	51.275	5.1E-09
N/V [1]	1.532	0.692
Lasix	-3.258	0.538
Slow K	1.461	0.788
Beta Blockers	-7.483	0.028
Aspirin	3.593	0.199
Age	-0.238	0.292
Model p-value	0.0617	
Multiple r-squared [3]	0.108	10.8%

[1] N/V = heterozygous genotype. [2] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [3] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [4] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.27: Results of the linear model showing the influence of variant 11 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [4]	P-value
Intercept [2]	51.415	4.8E-09
N/V [1]	1.72	0.649
Lasix	-3.278	0.536
Slow K	1.479	0.785
Beta Blockers	-7.573	0.027
Aspirin	3.585	0.199
Age	-0.243	0.284
Model p-value	0.0606	
Multiple r-squared [3]	0.109	10.9%

[1] N/V = heterozygous genotype. [2] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [3] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [4] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.28: Results of the linear model showing the influence of variant 12 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	52.53	2.4E-09
N/V [1]	-5.678	0.082
V/V [2]	4.223	0.594
Lasix	-3.77	0.472
Slow K	2.012	0.708
Beta Blockers	-6.587	0.05
Aspirin	3.959	0.152
Age	-0.25	0.27
Model p-value	0.0317	
Multiple r-squared [4]	0.137	13.7%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.29: Results of the linear model showing the influence of variant 13 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	49.562	5.8E-09
N/V [1]	7.088	0.01
V/V [2]	8.975	0.075
Lasix	-1.673	0.745
Slow K	-1.768	0.741
Beta Blockers	-8.142	0.015
Aspirin	3.142	0.245
Age	-0.249	0.256
Model p-value	0.0055	
Multiple r-squared [4]	0.175	17.5%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.30: Results of the linear model showing the influence of variant 14 on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Standard [5]	P-value
Intercept [3]	46.955	6.50E-08
N/V [1]	5.807	0.044
V/V [2]	8.059	0.026
Lasix	-1.063	0.839
Slow K	-0.988	0.854
Beta Blockers	-8.192	0.014
Aspirin	3.558	0.193
Age	-0.227	0.302
Model p-value	0.0118	
Multiple r-squared [4]	0.159	15.9%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.31: Results of the linear model showing the influence of all the *CYP2C9* variants (1-12) on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Std [5]	P-value
Intercept [3]	59.1474	6.32E-09
1-N/V [1]	-14.295	0.05457
1-V/V [2]	-22.9568	0.1969
2-N/V [1]	-0.7984	0.78545
2-V/V [2]	15.247	0.12107
3-N/V [1]	-5.1644	0.30373
4-N/V [1]	2.129	0.79409
4-V/V [2]	9.7753	0.6336
5-N/V [1]	-0.4453	0.9559
5-V/V [2]	-18.3812	0.25286
6-N/V [1]	-9.1005	0.12782
6-V/V [2]	22.105	0.06138
7-N/V [1]	7.6084	0.17438
7-V/V [2]	NA	NA
8-N/V [1]	0.4323	0.91455
8-V/V [2]	-9.4941	0.19506
9-N/V [1]	-4.7732	0.4911
10-N/V [1]	-3.4235	0.80967
11-N/V [1]	4.0242	0.76909
12-N/V [1]	8.9641	0.22087
12-V/V [2]	26.8789	0.08832
Lasix	-5.8703	0.26884
Slow K	5.7497	0.3071
Beta Blockers	-11.2745	0.00222
Aspirin	3.454	0.24394
Age	-0.3911	0.10842
Model p-value	0.02373	
Multiple r-squared [4]	0.3397	33.9%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.32: Results of the linear model showing the influence of both *VKORC1* variants (13-14) on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Std [5]	P-value
Intercept [3]	47.9948	4.06E-08
13-N/V [1]	5.3134	0.1245
13-V/V [2]	6.9137	0.2836
14-N/V [1]	2.7914	0.4213
14-V/V [2]	3.235	0.4999
Lasix	-1.1177	0.8302
Slow K	-2.0321	0.7058
Beta Blockers	-8.3106	0.0137
Aspirin	3.2553	0.2325
Age	-0.2415	0.2742
Model p-value	0.01468	
Multiple r-squared [4]	0.1804	18%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.33: Results of the linear model showing the influence of all 14 variants on warfarin dosage accounting for the four concomitant medications and age

Coefficients	Estimated Std [5]	P-value
Intercept [3]	56.61376	9.42E-09
1-N/V [1]	-15.297	0.040004
1-V/V [2]	-29.45639	0.082857
2-N/V [1]	0.53888	0.846085
2-V/V [2]	23.37698	0.014754
3-N/V [1]	-2.3126	0.631922
4-N/V [1]	5.83582	0.475047
4-V/V [2]	1.01425	0.960362
5-N/V [1]	-1.8898	0.812513
5-V/V [2]	-21.03899	0.192167
6-N/V [1]	-11.3522	0.048061
6-V/V [2]	20.08085	0.074411
7-N/V [1]	9.64084	0.074009
7-V/V [2]	-0.08806	0.981361
8-N/V [1]	-8.5237	0.21354
8-V/V [2]	-0.28036	0.966137
9-N/V [1]	-15.51771	0.25887
10-N/V [1]	14.60764	0.267305
11-N/V [1]	10.36136	0.150849
12-N/V [1]	32.85769	0.02807
13-N/V [1]	3.02794	0.387639
13-V/V [2]	-3.58363	0.587666
14-N/V [1]	6.24395	0.090399
14-V/V [2]	13.61741	0.007961
Lasix	-3.22576	0.518204
Slow K	2.19805	0.684805
Beta Blockers	-11.74082	0.000811
Aspirin	3.97906	0.154831
Age	-0.53102	0.022783
Model p-value	0.00127	
Multiple r-squared [4]	0.4526	45.3%

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = warfarin dosage in mg/week if the patient is 0 years old, is not taking any of the concomitant medications and is homozygous for the wild-type alleles. [4] Multiple r-squared x 100 = Percentage of warfarin dosage variability the coefficients in the table account for. [5] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Haplo.stats Results for warfarin dosage analysis

Tables 5.34 – 5.43 show all the haplo.stats results used to determine the influence of certain allele combinations on warfarin dosage. Each Haplo.stats result has two tables. The first represents the possible allele combinations, for the different variants, found within the patients. The second shows the estimated standard and p-values for each allele combination. The intercept in each table represents the warfarin dosage (in mg/week) in a patient with the haplotype base or wild type allele combination (i.e. the allele combination contains all the wild type alleles). The allele combination frequency is the frequency of that particular allele combination in the patient sample group. The rare allele combinations are represented by an asterisk (*) in the different variant columns because there are too many possible alleles to be listed.

Table 5.34: Haplo.stats results using all 12 *CYP2C9* variants

Allele combination	1	2	3	4	5	6	7	8	9	10	11	12	Allele combination Freq
1	A	A	C	T	T	T	G	G	C	T	C	T	0.072
2	G	A	C	C	C	T	G	A	C	T	C	C	0.1092
3	G	A	C	T	T	A	C	G	C	T	C	C	0.0921
4	G	A	C	T	T	T	G	A	C	C	T	C	0.0623
5	G	A	C	T	T	T	G	G	C	T	C	C	0.0369
6	G	A	T	T	T	T	G	A	C	T	C	C	0.0241
7	G	G	C	T	T	T	G	A	C	T	C	C	0.1317
Rare	*	*	*	*	*	*	*	*	*	*	*	*	0.1419
Haplo base [1]	**G**	**A**	**C**	**T**	**T**	**T**	**G**	**A**	**C**	**T**	**C**	**C**	**0.3299**

Allele combination	Estimated Standard [2]	P-value
Intercept	43.505	0.0000
1	-6.542	0.0756
2	0.836	0.7660
3	-0.925	0.7380
4	-0.615	0.8280
5	-0.499	0.6780
6	-1.982	0.0000
7	2.129	0.4360
Rare	-3.342	0.1790

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.35: Haplo.stats results using *CYP2C9* variants 1 – 6

Allele combination	1	2	3	4	5	6	Allele combination Freq
1	A	A	C	T	T	T	0.1054
2	G	A	C	C	C	T	0.1227
3	G	A	C	T	T	A	0.1074
4	G	G	C	T	T	T	0.1435
Rare	*	*	*	*	*	*	0.0747
Haplo Base [1]	**G**	**A**	**C**	**T**	**T**	**T**	**0.4463**

Allele combination	Estimated Standard [2]	P-value
Intercept	43.067	0
1	-6.035	0.0368
2	0.952	0.6984
3	-1.206	0.6526
4	2.434	0.3103
Rare	-3.28	0.2366

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.36: Haplo.stats results using *CYP2C9* variants 2 – 7

Allele combination	2	3	4	5	6	7	Allele combination Freq
1	A	C	C	C	T	G	0.1136
2	A	C	T	T	A	C	0.0909
3	A	T	T	T	T	G	0.0238
4	G	C	T	T	T	G	0.1545
Rare	*	*	*	*	*	*	0.0631
Haplo Base [1]	**A**	**C**	**T**	**T**	**T**	**G**	**0.5541**

Allele combination	Estimated Standard [2]	P-value
Intercept	40.791	0
1	2.275	0.412
2	-0.146	0.959
3	-0.254	0.475
4	2.148	0.393
Rare	0.35	0.866

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.37: Haplo.stats results using *CYP2C9* variants 3 – 8

Allele combination	3	4	5	6	7	8	Allele combination Freq
1	C	C	C	T	G	A	0.1148
2	C	T	T	A	C	G	0.0915
3	C	T	T	T	G	G	0.161
4	T	T	T	T	G	A	0.0323
Rare	*	*	*	*	*	*	0.0549
Haplo Base [1]	**C**	**T**	**T**	**T**	**G**	**A**	**0.5455**

Allele combination	Estimated Standard [2]	P-value
Intercept	44.268	0
1	0.416	0.887
2	-0.886	0.7713
3	-5.888	0.0257
4	-3.248	0.1029
Rare	-1.081	0.7871

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.38: Haplo.stats results using *CYP2C9* variants 4 – 9

Allele combination	4	5	6	7	8	9	Allele combination Freq
1	C	C	T	G	A	C	0.1077
2	T	T	A	C	G	C	0.0914
3	T	T	T	G	G	C	0.1611
Rare	*	*	*	*	*	*	0.0731
Haplo Base [1]	**T**	**T**	**T**	**G**	**A**	**C**	**0.5666**

Allele combination	Estimated Standard [2]	P-value
Intercept	44.114	0
1	0.577	0.854
2	-0.885	0.776
3	-5.931	0.028
Rare	-1.289	0.741

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.39: Haplo.stats results using *CYP2C9* variants 5 – 10

Allele combination	5	6	7	8	9	10	Allele combination Freq
1	C	T	G	A	C	T	0.1205
2	T	A	C	G	C	T	0.0932
3	T	T	G	A	C	C	0.0636
4	T	T	G	G	C	T	0.1615
Rare	*	*	*	*	*	*	0.0615
Haplo Base [1]	**T**	**T**	**G**	**A**	**C**	**T**	**0.4996**

Allele combination	Estimated Standard [2]	P-value
Intercept	44.601	0
1	0.147	0.9611
2	-1.638	0.5922
3	-1.326	0.6482
4	-6.148	0.012
Rare	-1.618	0.6337

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.40: Haplo.stats results using *CYP2C9* variants 6 – 11

Allele combination	6	7	8	9	10	11	Allele combination Freq
1	A	C	G	C	T	C	0.0997
2	T	G	A	C	C	T	0.0636
3	T	G	G	C	T	C	0.1561
Rare	*	*	*	*	*	*	0.0596
Haplo Base [1]	**T**	**G**	**A**	**C**	**T**	**C**	**0.6209**

Allele combination	Estimated Standard [2]	P-value
Intercept	44.68	0
1	-1.64	0.5495
2	-1.39	0.6204
3	-6.08	0.0147
Rare	-2.54	0.3525

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.41: Haplo.stats results using *CYP2C9* variants 7 – 12

Allele combination	7	8	9	10	11	12	Allele combination Freq
1	C	G	C	T	C	C	0.0986
2	G	A	C	C	T	C	0.0596
3	G	G	C	T	C	C	0.071
4	G	G	C	T	C	T	0.097
Rare	*	*	*	*	*	*	0.0629
Haplo Base [1]	**G**	**A**	**C**	**T**	**C**	**C**	**0.6109**

Allele combination	Estimated Standard [2]	P-value
Intercept	44.429	0
1	-1.307	0.6454
2	-0.892	0.7323
3	-6.21	0.0514
4	-6.339	0.0322
Rare	0.194	0.9531

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.42: Haplo.stats results using *VKORC1* variants 13 and 14

Allele combination	13	14	Allele combination Freq
1	C	A	0.177
2	T	A	0.255
Haplo Base [1]	**C**	**G**	**0.568**

Allele combination	Estimated Standard [2]	P-value
Intercept	38.84	0
1	2.21	0.3641
2	4.62	0.0315

[1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Table 5.43: Haplo.stats results using 13 of the *CYP2C9* and *VKORC1* variants*

Allele combination	1	2	3	4	5	6	7	8	10	11	12	13	14	Allele combination Freq
1	A	A	C	T	T	T	G	G	T	C	T	C	A	0.0305
2	A	A	C	T	T	T	G	G	T	C	T	C	G	0.0308
3	G	A	C	C	C	T	G	A	T	C	C	C	G	0.0822
4	G	A	C	T	T	A	C	G	T	C	C	C	G	0.0428
5	G	A	C	T	T	A	C	G	T	C	C	T	A	0.0341
6	G	A	C	T	T	T	G	A	T	C	C	C	A	0.0441
7	G	A	C	T	T	T	G	A	T	C	C	T	A	0.1266
8	G	A	C	T	T	T	G	G	T	C	C	C	G	0.0313
9	G	G	C	T	T	T	G	A	T	C	C	C	G	0.1021
Rare	*	*	*	*	*	*	*	*	*	*	*	*	*	0.2835
Haplo Base [1]	**G**	**A**	**C**	**T**	**T**	**T**	**G**	**A**	**T**	**C**	**C**	**C**	**G**	**0.1919**

Allele combination	Estimated Standard [2]	P-value
Intercept	33.298	0.0000
1	-7.433	0.0000
2	3.081	0.0000
3	1.011	0.6040
4	6.758	0.0000
5	-0.391	0.0628
6	0.343	0.2620
7	11.592	0.0000
8	3.802	0.0000
9	6.11	0.0206
Rare	6.655	0.0007

* Variant 9 had to be excluded from the analysis because the numbers of patients with the variant allele were too small. [1] Haplo base = allele combination with all the wild type alleles. [2] In the Estimated Standard column: a positive value = increase in warfarin dosage in mg/week; a negative value = decrease in warfarin dosage in mg/week.

Appendix L

Statistical Values for the Pregnancy Analyses

All p-values highlighted in the tables are smaller than 0.05 and were considered significant.

Bar Graphs

The size of the bars in the graphs, shown in figures 5.1 and 5.2, represent the number of patients with that particular genotype. Some of these graphs lack the 3rd genotype because of the small numbers of patients with that genotype. These bar graphs are based on the outcome of the patients' first pregnancy (n=108). The values on the Y-axis represent the frequencies of normal and poor pregnancies for the genotype represented on the X-axis. In the graphs the smallest bars are always the homozygous variant genotype (V/V).

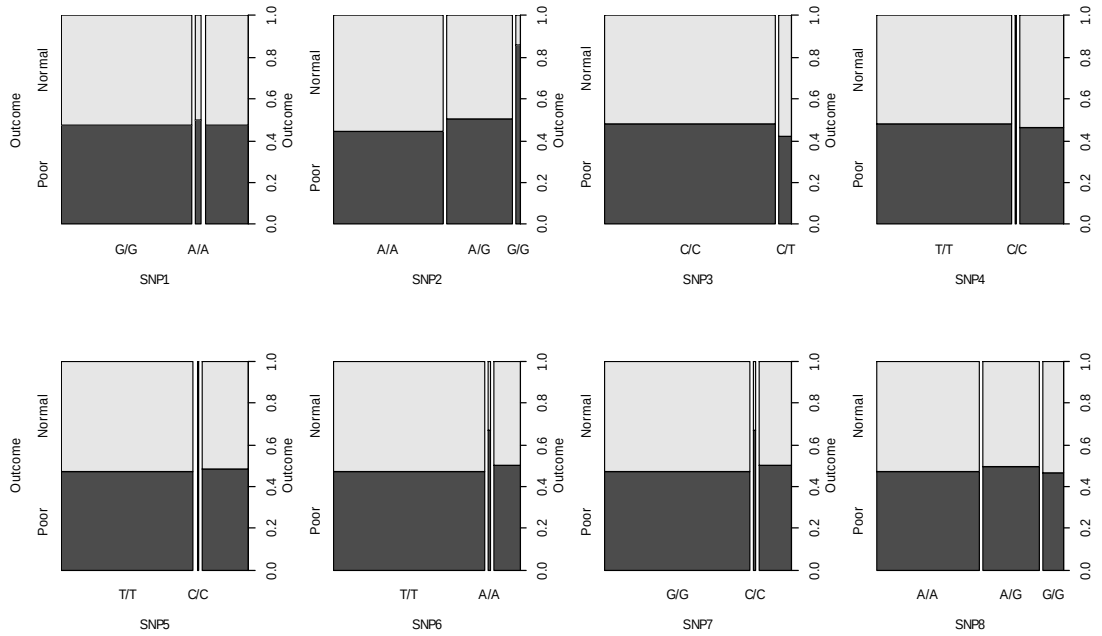


Figure 5.1: Bar Graphs for variants 1 – 8 showing their influence on pregnancy outcome

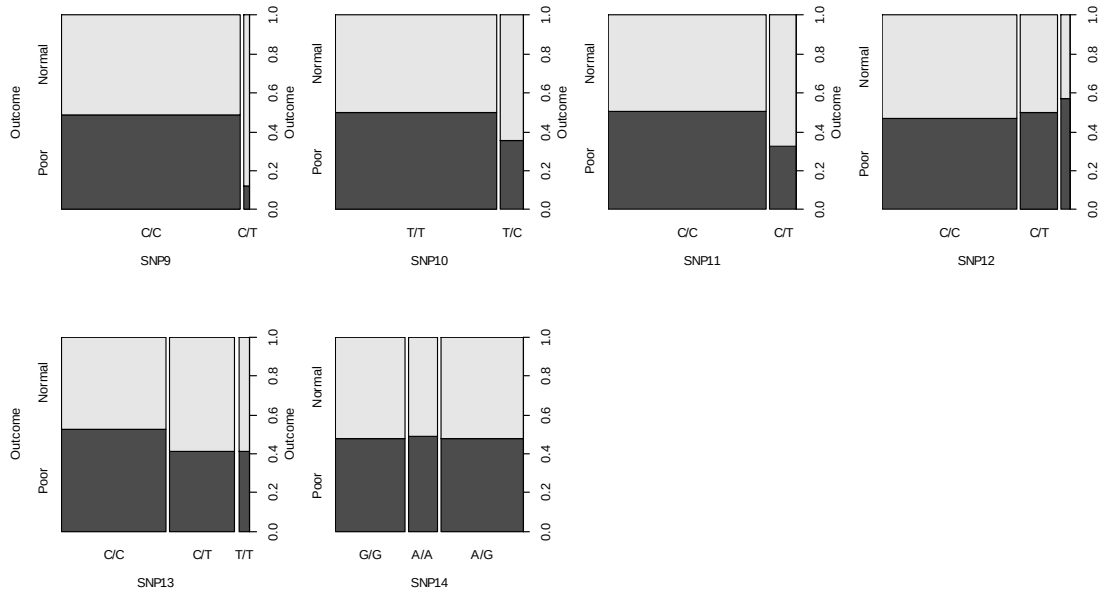


Figure 5.2: Bar Graphs for variants 9 – 14 showing their influence on pregnancy outcome

Generalised linear model results for pregnancy outcome analysis

In these models the column labelled "value" represents the degree by which the factor (age, number of pregnancies, heparin, warfarin or variant) affects pregnancy outcome. Positive values represent an increase in the risk of having a poor pregnancy outcome. Negative values represent a decrease in the risk of having a poor pregnancy outcome. The intercept in these models show the risk of having a poor pregnancy outcome if the patients' age is 0, had one pregnancy, and was not taking warfarin or heparin during pregnancy.

Table 5.44: Generalised linear model result for age and number of pregnancies

Factors	Value	p-value
Intercept	-5.277	0.0000
No. Pregnancies	-0.323	0.0469
Age	0.219	0.0000

Intercept = risk of a poor pregnancy outcome if the patient is 0 years old and is having their 1st pregnancy. In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.45: Generalised linear model result for age, number of pregnancies, warfarin and heparin

Factors	Value [2]	p-Value
Intercept [1]	-5.834143	0.0000
No. Pregnancies	-0.302832	0.0827
Age	0.173328	0.0001
Heparin	-1.396958	0.0001
Warfarin	2.805595	0.0000

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy and is not taking heparin or warfarin. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.46: Results of the generalised linear model showing the influence of variant 1 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-6.111	0.0000
No. Pregnancies	-0.351	0.0547
Age	0.182	0.0001
Warfarin	2.867	0.0000
Heparin	-1.445	0.0001
N/V [1]	0.42	0.3113
V/V [2]	0.827	0.4329

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.47: Results of the generalised linear model showing the influence of variant 2 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-5.878	0.0000
No. Pregnancies	-0.257	0.1622
Age	0.159	0.0007
Warfarin	2.879	0.0000
Heparin	-1.298	0.0008
N/V [1]	0.556	0.1297
V/V [2]	3.119	0.0382

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.48: Results of the generalised linear model showing the influence of variant 3 on pregnancy outcome

Factors	Value [3]	p-value
Intercept [2]	-5.7813	0.0000
No. Pregnancies	-0.3003	0.0857
Age	0.1717	0.0001
Warfarin	2.8112	0.0000
Heparin	-1.39	0.0001
N/V [1]	-0.3026	0.6478

[1] N/V = heterozygous genotype. [2] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [3] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.49: Results of the generalised linear model showing the influence of variant 4 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-5.85853	0.0000
No. Pregnancies	-0.30184	0.0855
Age	0.175363	0.0001
Warfarin	2.772856	0.0000
Heparin	-1.37856	0.0001
N/V [1]	-0.11292	0.7878
V/V [2]	24.27095	0.9998

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.50: Results of the generalised linear model showing the influence of variant 5 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-5.931135	0.0000
No Pregnancies	-0.298992	0.0873
Age	0.176081	0.0001
Warfarin	2.756591	0.0000
Heparin	-1.366261	0.0001
N/V [1]	0.080185	0.8436
V/V [2]	25.255988	0.9999

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.51: Results of the generalised linear model showing the influence of variant 6 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-5.844	0.0000
No. Pregnancies	-0.308	0.0823
Age	0.174	0.0001
Warfarin	2.819	0.0000
Heparin	-1.412	0.0001
N/V [1]	0.003	0.9944
V/V [2]	-0.523	0.7208

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.52: Results of the generalised linear model showing the influence of variant 7 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-5.856	0.0000
No. Pregnancies	-0.307	0.0817
Age	0.174	0.0001
Warfarin	2.819	0.0000
Heparin	-1.412	0.0000
N/V [1]	0.033	0.9420
V/V [2]	-0.517	0.7238

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.53: Results of the generalised linear model showing the influence of variant 8 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-6.117	0.0000
No. Pregnancies	-0.313	0.0762
Age	0.179	0.0001
Warfarin	2.803	0.0000
Heparin	-1.418	0.0001
N/V [1]	0.463	0.2297
V/V [2]	0.126	0.8240

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.54: Results of the generalised linear model showing the influence of variant 9 on pregnancy outcome

Factors	Value [3]	p-value
Intercept [2]	-5.843	0.0000
No. Pregnancies	-0.318	0.0711
Age	0.176	0.0001
Warfarin	2.78	0.0000
Heparin	-1.342	0.0002
N/V [1]	-1.415	0.2397

[1] N/V = heterozygous genotype. [2] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [3] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.55: Results of the generalised linear model showing the influence of variant 10 on pregnancy outcome

Factors	Value [3]	p-value
Intercept [2]	-5.763	0.0000
No. Pregnancies	-0.305	0.0823
Age	0.174	0.0001
Warfarin	2.854	0.0000
Heparin	-1.425	0.0001
N/V [1]	-0.795	0.1290

[1] N/V = heterozygous genotype. [2] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [3] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.56: Results of the generalised linear model showing the influence of variant 11 on pregnancy outcome

Factors	Value [3]	p-value
Intercept [2]	-5.702	0.0000
No. Pregnancies	-0.278	0.1109
Age	0.171	0.0001
Warfarin	2.817	0.0000
Heparin	-1.395	0.0001
N/V [1]	-0.832	0.0916

[1] N/V = heterozygous genotype. [2] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [3] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.57: Results of the generalised linear model showing the influence of variant 12 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-6.231	0.0000
No. Pregnancies	-0.381	0.0376
Age	0.185	0.0001
Warfarin	2.957	0.0000
Heparin	-1.445	0.0001
N/V [1]	0.423	0.3483
V/V [2]	1.532	0.1010

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.58: Results of the generalised linear model showing the influence of variant 13 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-5.709	0.0000
No. Pregnancies	-0.351	0.0457
Age	0.186	0.0000
Warfarin	2.883	0.0000
Heparin	-1.511	0.0000
N/V [1]	-0.741	0.0440
V/V [2]	-1.407	0.0490

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or

warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Table 5.59: Results of the generalised linear model showing the influence of variant 14 on pregnancy outcome

Factors	Value [4]	p-value
Intercept [3]	-5.788	0.0000
No. Pregnancies	-0.355	0.0468
Age	0.184	0.0001
Warfarin	2.889	0.0000
Heparin	-1.479	0.0001
N/V [1]	-0.305	0.4355
V/V [2]	-0.703	0.1742

[1] N/V = heterozygous genotype; [2] V/V = homozygous variant genotype. [3] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [4] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome.

Interaction Models

These models show the influence of the 14 *CYP2C9* and *VKORC1* variants on pregnancy outcome when they interact with warfarin, adjusting for the influence of the environmental factors identified in section 3.3.1. Each result has two sections. The first section describes the different factors (age, number of pregnancies, heparin, warfarin and variants), their level of influence on pregnancy outcome and their p-values. In this section the factor shown as Warfarin: V represents the interaction between warfarin and that variant. The second section shows the overall value of influence of a particular allele on pregnancy outcome when warfarin is and is not being taken during pregnancy. N represents the wild type allele and V represents the variant allele. If a patient has a normal allele and is taking warfarin the degree of influence is determined by the value shown when warfarin is taken (in the first section). If the patient is not taking warfarin and has the normal allele they will have no value of influence on pregnancy outcome. If the patient is not on warfarin but they have the variant allele their degree of influence is determined by the value of the variant

allele alone. If the patient is on warfarin and they have the variant allele their degree of influence is determined by the sum of the value of warfarin alone, the allele alone and the interaction of the allele and warfarin. I was unable to obtain results for variants 6, 9, 10, 11 and 13 because the models gave large statistical errors.

Table 5.60: Interaction model showing the influence of variant 1 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-6.059	0.0000
Pregnancy	-0.367	0.0487
Age	0.184	0.0001
Heparin	-1.455	0.0001
Warfarin	2.766	0.0000
Variant Allele	0.22	0.7443
Warfarin : V [4]	0.252	0.7270
	N [3]	**V** [4]
Warfarin (Yes)	2.766	3.238
Warfarin (No)	0	0.220

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.61: Interaction model showing the influence of variant 2 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-7.484	0.0000
Pregnancy	-0.385	0.0329
Age	0.184	0.0001
Heparin	-1.519	0.0000
Warfarin	4.182	0.0000
Variant Allele [4]	2.115	0.0111
Warfarin : V [4]	-1.509	0.0845
	N [3]	**V** [4]
Warfarin (Yes)	4.182	4.788
Warfarin (No)	0	2.115

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive

value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.62: Interaction model showing the influence of variant 3 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-6.304	0.0000
Pregnancy	-0.351	0.0564
Age	0.184	0.0001
Heparin	-1.554	0.0000
Warfarin	3.234	0.0000
Variant Allele [4]	2.766	0.0503
Warfarin : V [4]	-3.51	0.0171
	N [3]	**V** [4]
Warfarin (Yes)	3.234	2.490
Warfarin (No)	0	2.766

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.63: Interaction model showing the influence of variant 4 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-5.746	0.0000
Pregnancy	-0.329	0.0618
Age	0.18	0.0001
Heparin	-1.443	0.0001
Warfarin	2.558	0.0000
Variant Allele [4]	-1.112	0.3010
Warfarin : V [4]	1.311	0.2434
	N [3]	**V** [4]
Warfarin (Yes)	2.558	2.757
Warfarin (No)	0	-1.112

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.64: Interaction model showing the influence of variant 5 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-5.787	0.0000
Pregnancy	-0.331	0.0595
Age	0.182	0.0001
Heparin	-1.449	0.0001
Warfarin	2.485	0.0000
Variant Allele [4]	-1.112	0.2989
Warfarin : V [4]	1.586	0.1555
	N [3]	V [4]
Warfarin (Yes)	2.485	2.959
Warfarin (No)	0	-1.112

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.65: Interaction model showing the influence of variant 7 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-5.955	0.0000
Pregnancy	-0.309	0.0801
Age	0.172	0.0001
Heparin	-1.406	0.0001
Warfarin	3.017	0.0000
Variant Allele [4]	0.763	0.4131
Warfarin : V [4]	-0.935	0.3471
	N [3]	**V** [4]
Warfarin (Yes)	3.017	2.845
Warfarin (No)	0	0.763

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.66: Interaction model showing the influence of variant 8 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-5.969	0.0000
Pregnancy	-0.313	0.0764
Age	0.177	0.0001
Heparin	-1.408	0.0001
Warfarin	2.763	0.0000
Variant Allele [4]	0.119	0.8353
Warfarin : V [4]	0.076	0.8998
	N [3]	**V** [4]
Warfarin (Yes)	2.763	2.958
Warfarin (No)	0	0.119

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.67: Interaction model showing the influence of variant 12 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-6.204	0.0000
Pregnancy	-0.362	0.0486
Age	0.181	0.0001
Heparin	-1.444	0.0001
Warfarin	2.979	0.0000
Variant Allele [4]	0.646	0.2594
Warfarin : V [4]	-0.083	0.8964
	N [3]	**V** [4]
Warfarin (Yes)	2.979	3.542
Warfarin (No)	0	0.646

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Table 5.68: Interaction model showing the influence of variant 14 on pregnancy outcome

Factors	Value [2]	p-Value
Intercept [1]	-5.563	0.0000
Pregnancy	-0.443	0.0176
Age	0.207	0.0000
Heparin	-1.521	0.0000
Warfarin	1.922	0.0018
Variant Allele [4]	-1.357	0.0201
Warfarin : V [4]	1.397	0.0188
	N [3]	**V** [4]
Warfarin (Yes)	1.922	1.962
Warfarin (No)	0	-1.357

[1] Intercept = risk of a poor pregnancy outcome if the patient is 0 years old, is having their 1st pregnancy, is not taking heparin or warfarin and is homozygous for the wild-type alleles. [2] In the value column: a positive value = increased risk of having a poor pregnancy outcome; a negative value = decreased risk of having a poor pregnancy outcome. [3] N = wild-type allele, [4] V = variant allele. Warfarin (yes) = warfarin taken during pregnancy; Warfarin (no) = warfarin not taken during pregnancy.

Haplo.stats results for pregnancy outcome analysis

These haplo.stats results show the influence of certain allele combinations on pregnancy outcome when warfarin is and is not taken during pregnancy, respectively. Each haplo.stats result contains two sections. In the top section, the pool allele combination frequency refers to the normal and poor outcome frequencies together. The haplo-score represents the influence of that particular allele combination on pregnancy outcome. Positive values result in an increase in the risk of having a poor pregnancy outcome. Negative values result in a decrease in having a poor pregnancy outcome. The p-values or sim p-values next to each allele combination is the p-value for that particular allele combination. The allele combinations in bold are the normal alleles, which are not always present in the analysis. The p-value in the bottom section is the probability value for the entire analysis.

Table 5.69: Haplo.stats result using variants 1-4 in pregnancies on warfarin

Allele combination	1	2	3	4	Haplo-Score	p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
2	G	A	C	C	-0.8718	0.3833	0.0905	0.1166	0.0662
5	A	A	C	C	-0.5353	0.5924	0.6254	0.6481	0.6113
4	A	A	C	T	0.5638	0.5729	0.1173	0.0988	0.1315
8	A	G	C	C	0.5850	0.5586	0.1173	0.1019	0.1255
1	**G**	**A**	**C**	**T**	**NA**	**NA**	**0.0000**	**0.0000**	**0.0000**
3	G	G	C	C	NA	NA	0.0051	0.0000	0.0127
6	A	A	T	C	NA	NA	0.0272	0.0198	0.0331
7	A	G	C	T	NA	NA	0.0004	0.0012	0.0001
9	A	G	T	C	NA	NA	0.0170	0.0136	0.0195

Model:	
Global-Stat	1.7583
d.f	4
p-Value	0.7801

The allele combination in bold = wild-type alleles

Table 5.70: Haplo.stats result using variants 1-4 in pregnancies off warfarin

Allele combination	1	2	3	4	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
6	A	A	C	C	-1.4776	0.1680	0.4859	0.5085	0.2500
5	A	A	C	T	-0.0869	1.0000	0.1342	0.1351	0.1250
1	G	A	C	C	0.6007	0.5880	0.1477	0.1395	0.2500
9	A	G	C	C	1.3390	0.2800	0.1842	0.1666	0.3750
2	G	A	T	C	NA	NA	0.0000	NA	NA
3	G	G	C	C	NA	NA	0.0237	0.0233	0.0000
4	G	G	T	C	NA	NA	0.0116	0.0129	NA
7	A	A	T	C	NA	NA	0.0128	0.0142	NA
8	A	G	C	T	NA	NA	0.0000	0.0000	NA

Model:	
Global-Stat	7.4808
d.f	4
p-Value	0.1126

Table 5.71: Haplo.stats result using variants 2-5 in pregnancies on warfarin

Allele combination	2	3	4	5	Haplo-Score	p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
3	A	C	C	C	-1.5659	0.1174	0.6962	0.7688	0.6374
1	**A**	**C**	**T**	**T**	**0.5558**	**0.5784**	**0.1154**	**0.0954**	**0.1316**
6	G	C	C	C	0.5953	0.5517	0.1200	0.0979	0.1389
2	A	C	C	T	NA	NA	0.0221	NA	0.0395
4	A	T	C	C	NA	NA	0.0266	0.0191	0.0336
5	G	C	T	T	NA	NA	0.0023	0.0046	0.0000
7	G	T	C	C	NA	NA	0.0175	0.0142	0.0190

Model:	
Global-Stat	3.3202
d.f	3
p-Value	0.3449

The allele combination in bold = wild-type alleles

Table 5.72: Haplo.stats result using variants 2-5 in pregnancies off warfarin

Allele combination	2	3	4	5	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
3	A	C	C	C	-0.8224	0.5240	0.6154	0.6291	0.5000
1	**A**	**C**	**T**	**T**	**-0.0869**	**0.9640**	**0.1342**	**0.1351**	**0.1250**
6	G	C	C	C	1.2539	0.2650	0.2138	0.1953	0.3750
2	A	C	C	T	NA	NA	0.0122	0.0135	NA
4	A	T	C	C	NA	NA	0.0187	0.0196	NA
5	G	C	T	T	NA	NA	0.0000	0.0000	NA
7	G	T	C	C	NA	NA	0.0057	0.0074	NA

Model:	
Global-Stat	1.7917
d.f	3
p-Value	0.6167

The allele combination in bold = wild-type alleles

Table 5.73: Haplo.stats result using variants 3-6 in pregnancies on warfarin

Allele combination	3	4	5	6	Haplo-Score	p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
5	C	C	C	T	-0.7833	0.4334	0.1490	0.1722	0.1297
6	C	C	C	A	-0.5401	0.5891	0.6672	0.6945	0.6466
2	C	T	T	A	0.5652	0.5719	0.1177	0.1000	0.1316
8	T	C	C	A	0.6803	0.4963	0.0379	0.0222	0.0494
1	**C**	**T**	**T**	**T**	**NA**	**NA**	**0.0000**	**NA**	**0.0000**
3	C	C	T	T	NA	NA	0.0138	NA	0.0249
4	C	C	T	A	NA	NA	0.0082	NA	0.0145

Model:	
Global-Stat	3.1037
d.f	4
p-Value	0.5406

The allele combination in bold = wild-type alleles

Table 5.74: Haplo.stats result using variants 3-6 in pregnancies off warfarin

Allele combination	3	4	5	6	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
1	C	T	T	A	-0.0869	0.8610	0.1341	0.1351	0.1250
4	C	C	C	A	0.7036	0.5970	0.7927	0.7838	0.8750
2	C	C	T	A	NA	NA	0.0122	0.0135	NA
3	C	C	C	T	NA	NA	0.0366	0.0405	NA
5	T	C	C	A	NA	NA	0.0244	0.0270	NA

Model:	
Global-Stat	0.85941
d.f	2
p-Value	0.6507

Table 5.75: Haplo.stats result using variants 4-7 in pregnancies on warfarin

Allele combination	4	5	6	7	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
5	C	C	T	G	-1.7033	0.0940	0.1257	0.1833	0.0797
7	C	C	A	C	-0.2688	0.7920	0.7052	0.7167	0.6966
2	T	T	A	C	0.5652	0.5900	0.1177	0.1000	0.1316
1	**T**	**T**	**T**	**G**	**NA**	**NA**	**0.0000**	**NA**	**0.0000**
3	C	T	T	G	NA	NA	0.0140	NA	0.0255
4	C	T	A	C	NA	NA	0.0080	NA	0.0139
6	C	C	T	C	NA	NA	0.0294	NA	0.0526

Model:	
Global-Stat	8.1166
d.f	3
p-Value	0.0437

The allele combination in bold = wild-type alleles

Table 5.76: Haplo.stats result using variants 4-7 in pregnancies off warfarin

Allele combination	4	5	6	7	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
2	T	T	A	C	-1.0299	0.4850	0.1139	0.1241	0.0000
6	C	C	A	C	-0.5257	0.6820	0.7886	0.7948	0.7500
1	T	T	A	G	NA	NA	0.0203	0.0110	0.1250
3	C	T	A	C	NA	NA	0.0122	0.0135	NA
4	C	C	T	G	NA	NA	0.0366	0.0405	NA
5	C	C	A	G	NA	NA	0.0285	0.0160	0.1250

Model:	
Global-Stat	3.0579
d.f	2
p-Value	0.2168

Table 5.77: Haplo.stats result using variants 5-8 in pregnancies on warfarin

Allele combination	5	6	7	8	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
4	C	T	G	G	-1.6325	0.1160	0.1282	0.1833	0.0765
7	C	A	C	A	-0.4592	0.6710	0.5605	0.5833	0.5501
8	C	A	C	G	0.2907	0.7730	0.1422	0.1333	0.1497
2	T	A	C	A	0.8908	0.4160	0.1282	0.1000	0.1423
1	T	T	G	G	NA	NA	0.0115	NA	0.0287
3	T	A	C	G	NA	NA	0.0000	0.0000	0.0000
5	C	T	C	A	NA	NA	0.0099	NA	0.0181
6	C	T	C	G	NA	NA	0.0196	NA	0.0346

Model:	
Global-Stat	8.0229
d.f	4
p-Value	0.0907

Table 5.78: Haplo.stats result using variants 5-8 in pregnancies off warfarin

Allele combination	5	6	7	8	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
2	T	A	C	A	-1.1377	0.5640	0.1252	0.1392	NA
7	C	A	C	A	-0.7546	0.4730	0.5943	0.6041	0.5000
8	C	A	C	G	0.3989	0.9070	0.1951	0.1892	0.2500
1	T	A	G	A	NA	NA	0.0211	0.0095	0.1250
3	T	A	C	G	NA	NA	0.0000	NA	NA
4	C	T	G	G	NA	NA	0.0366	0.0405	NA
5	C	A	G	A	NA	NA	0.0155	0.0176	NA
6	C	A	G	G	NA	NA	0.0122	NA	0.1250

Model:	
Global-Stat	4.0127
d.f	3
p-Value	0.2601

Table 5.79: Haplo.stats result using variants 6-9 in pregnancies on warfarin

Allele combination	6	7	8	9	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
1	T	G	G	C	-1.2534	0.2290	0.1397	0.1833	0.1053
6	A	C	G	C	0.2727	0.7990	0.1417	0.1333	0.1483
4	A	C	A	C	0.5254	0.5820	0.6745	0.6500	0.6938
2	T	C	A	C	NA	NA	0.0093	NA	0.0167
3	T	C	G	C	NA	NA	0.0201	NA	0.0359
5	A	C	A	T	NA	NA	0.0147	0.0333	NA

Model:	
Global-Stat	1.8403
d.f	3
p-Value	0.6062

Table 5.80: Haplo.stats result using variants 6-9 in pregnancies off warfarin

Allele combination	6	7	8	9	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
4	A	C	A	C	-1.2485	0.1810	0.6951	0.7162	0.5000
6	A	C	G	C	0.3989	0.8450	0.1951	0.1892	0.2500
1	T	G	G	C	NA	NA	0.0366	0.0405	NA
2	A	G	A	C	NA	NA	0.0366	0.0270	0.1250
3	A	G	G	C	NA	NA	0.0122	NA	0.1250
5	A	C	A	T	NA	NA	0.0244	0.0270	NA

Model:	
Global-Stat	2.4271
d.f	2
p-Value	0.2972

Table 5.81: Haplo.stats result using variants 7-10 in pregnancies on warfarin

Allele combination	7	8	9	10	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
1	G	G	C	C	-1.2534	0.2530	0.1397	0.1833	0.1053
2	C	A	C	T	-1.1148	0.4410	0.0588	0.0833	0.0395
5	C	G	C	C	0.8906	0.4030	0.1618	0.1333	0.1842
3	C	A	C	C	1.3146	0.2400	0.6250	0.5667	0.6711
4	C	A	T	C	NA	NA	0.0147	0.0333	NA

Model:	
Global-Stat	7.0695
d.f	4
p-Value	0.1323

Table 5.82: Haplo.stats result using variants 7-10 in pregnancies off warfarin

Allele combination	7	8	9	10	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
4	C	A	C	C	-0.7989	0.2970	0.6283	0.6425	0.5000
7	C	G	C	C	0.3555	0.7810	0.2010	0.1953	0.2500
1	G	A	C	C	NA	NA	0.0424	0.0331	0.1250
2	G	G	C	C	NA	NA	0.0429	0.0344	0.1250
3	C	A	C	T	NA	NA	0.0610	0.0676	NA
5	C	A	T	C	NA	NA	0.0244	0.0270	NA
6	C	G	C	T	NA	NA	0.0000	0.0000	NA

Model:	
Global-Stat	0.74395
d.f	2
p-Value	0.6894

Table 5.83: Haplo.stats result using variants 8-11 in pregnancies on warfarin

Allele combination	8	9	10	11	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
1	A	C	T	T	-1.1148	0.4560	0.0588	0.0833	0.0395
4	G	C	C	C	-0.3232	0.7600	0.3015	0.3167	0.2895
2	A	C	C	C	1.3146	0.2430	0.6250	0.5667	0.6711
3	A	T	C	C	NA	NA	0.0147	0.0333	NA

Model:	
Global-Stat	5.1863
d.f	3
p-Value	0.1587

Table 5.84: Haplo.stats result using variants 8-11 in pregnancies off warfarin

Allele combination	8	9	10	11	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
2	A	C	C	C	-0.2813	0.8130	0.6707	0.6757	0.6250
5	G	C	C	C	0.9057	0.3750	0.2317	0.2162	0.3750
1	A	C	T	T	NA	NA	0.0610	0.0676	NA
3	A	T	C	C	NA	NA	0.0244	0.0270	NA
4	G	C	T	T	NA	NA	0.0000	0.0000	NA
6	G	C	C	T	NA	NA	0.0122	0.0135	NA

Model:	
Global-Stat	1.5203
d.f	2
p-Value	0.4676

Table 5.85: Haplo.stats result using variants 9-12 in pregnancies on warfarin

Allele combination	9	10	11	12	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
1	C	T	T	C	-1.1148	0.4640	0.0588	0.0833	0.0395
3	C	C	C	T	-0.0975	1.0000	0.0809	0.0833	0.0789
2	C	C	C	C	1.4460	0.2040	0.8456	0.8000	0.8816
4	T	C	C	C	NA	NA	0.0147	0.0333	NA

Model:	
Global-Stat	4.2835
d.f	3
p-Value	0.2324

Table 5.86: Haplo.stats result using variants 9-12 in pregnancies off warfarin

Allele combination	9	10	11	12	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
3	C	C	C	C	-0.6864	0.5510	0.7329	0.7470	0.6250
4	C	C	C	T	1.4237	0.1450	0.1695	0.1449	0.3750
1	C	T	T	C	NA	NA	0.0476	0.0503	NA
2	C	T	T	T	NA	NA	0.0134	0.0173	NA
5	C	C	T	C	NA	NA	0.0122	0.0135	NA
6	T	C	C	C	NA	NA	0.0244	0.0270	NA

Model:	
Global-Stat	2.6497
d.f	2
p-Value	0.2659

Table 5.87: Haplo.stats result using variants 13-14 in pregnancies on warfarin

Allele combination	13	14	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
2	C	A	-0.6223	0.5460	0.5515	0.5833	0.5263
3	T	G	0.1650	0.9250	0.2574	0.2500	0.2632
1	**C**	**G**	**0.6306**	**0.6380**	**0.1912**	**0.1667**	**0.2105**

Model:	
Global-Stat	0.50992
d.f	2
p-Value	0.7750

The allele combination in bold = wild-type alleles

Table 5.88: Haplo.stats result using variants 13-14 in pregnancies off warfarin

Allele combination	13	14	Haplo-Score	Sim p-Value	(Pool) Haplo. Freq	(Normal) Haplo. Freq	(Poor) Haplo. Freq
3	T	G	-1.8295	0.0830	0.2561	0.2838	NA
1	**C**	**G**	**-0.1777**	**1.0000**	**0.1463**	**0.1487**	**0.1250**
2	C	A	1.7420	0.0390	0.5976	0.5676	0.8750

Model:	
Global-Stat	3.7977
d.f	2
p-Value	0.1497

The allele combination in bold = wild-type alleles. The allele combination that has been highlighted = shows a significant sim p-value

Appendix M

This appendix contains the raw genotype and clinical data that was collected and used for this project. Referred in the first paragraph of page 44.

Genotype Data

Table 5.89: Genotype Data for the first 15 previously described CYP2C9 variants in the 100 Control Samples

Sample Number	2 - R144C	3 - I354K	4 - I359T	5 - D360E	6 - 818 delA	7 - L19I	8 - R150H	9 - H251R	10 - E272G	11 - R355W	12 - P489S	13 - L90P	14 - R125H	15 - S162X
1	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
2	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
3	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
4	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
5	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
6	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
7	C/C	A/A	T/T	C/G	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
8	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
9	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
10	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
11	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
12	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
13	C/C	A/A	T/T	C/G	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
14	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
15	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
16	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C

17	C/C	A/C	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
18	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
19	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
20	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
21	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
22	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
23	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
24	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
25	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
26	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
27	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
28	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
29	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
30	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
31	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
32	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
33	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/T	C/C	T/T	G/G	C/C
34	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
35	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
36	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
37	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
38	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
39	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
40	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
41	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
42	C/C	A/A	T/T	C/C	A/A	C/C	G/G	G/G	A/A	C/C	C/C	T/T	G/G	C/C
43	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
44	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
45	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
46	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
47	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
48	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
49	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C

50	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
51	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
52	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
53	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
54	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
55	C/C	A/A	T/T	C/C	A/A	C/C	G/G	G/G	A/A	C/C	C/C	T/T	G/G	C/C
56	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
57	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
58	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
59	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
60	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
61	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
62	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
63	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
64	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
65	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	T/T	C/C	T/T	G/G	C/C
66	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
67	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
68	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
69	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
70	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
71	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
72	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
73	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
74	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
75	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
76	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
77	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
78	C/C	A/A	T/T	C/C	A/A	C/C	G/G	G/G	A/A	C/C	C/C	T/T	G/G	C/C
79	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
80	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
81	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
82	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C

83	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
84	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
85	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
86	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
87	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
88	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
89	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
90	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
91	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
92	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
93	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
94	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
95	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
96	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
97	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
98	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
99	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
100	C/C	A/A	T/T	C/C	A/-	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
TOTALS	C = 200	A = 199	T = 200	C = 198	A = 199	C = 200	G = 184	A = 176	A = 200	C = 191	C = 200	T = 200	G = 200	C = 200
TOTALS	T = 0	C = 1	C = 0	G = 2	Del = 1	A = 0	A = 16	G = 24	G = 0	T = 9	T = 0	C = 0	A = 0	A = 0
	0	1	0	2	1	0	16	18	0	7	0	0	0	0
	0	0	0	0	0	0	0	3	0	1	0	0	0	0

In this table the variants that have been highlighted in blue are the variants for which some patients or controls were heterozygous or homozygous and were used in the analyses described in sections three to four. Heterozygotes are highlighted in orange and homozygotes in green.

Table 5.90: Genotype Data for variants 16 – 30 of the previously described CYP2C9 variants in the 100 Control Samples

Sample Number	16 - T299A	17 - P382S	18 - D397A	19 - Q454H	20 - G70R	21 - P30L	22 - N41D	23 - V76M	24 - E354K	25 - 353-362del	26 - T130R	27 - R150L	28 - Q214L	29 - P279T	30 - A477T
1	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
2	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
3	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
4	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
5	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
6	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
7	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
8	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
9	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
10	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
11	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
12	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
13	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
14	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
15	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
16	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
17	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
18	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
19	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
20	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
21	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
22	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
23	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
24	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
25	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
26	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
27	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
28	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G

29	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
30	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
31	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
32	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
33	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
34	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
35	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
36	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
37	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
38	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
39	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
40	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
41	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
42	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
43	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
44	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
45	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
46	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
47	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
48	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
49	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
50	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
51	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
52	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
53	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
54	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
55	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
56	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
57	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
58	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
59	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
60	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
61	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G

62	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
63	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
64	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
65	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
66	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
67	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
68	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
69	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
70	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
71	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
72	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
73	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
74	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
75	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
76	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
77	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
78	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
79	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
80	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
81	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
82	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
83	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
84	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
85	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
86	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
87	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
88	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
89	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
90	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
91	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
92	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
93	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
94	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G

95	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
96	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
97	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
98	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/A	A/A	C/C	G/G
99	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
100	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No Del	C/C	G/G	A/A	C/C	G/G
TOTALS	A = 200	C = 200	A = 200	G = 200	G = 200	C = 200	A = 200	G = 200	G = 200	No Del = 200	C = 200	G = 184	A = 200	C = 200	G = 200
	G = 0	T = 0	C = 0	C = 0	C = 0	T = 0	G = 0	A = 0	A = 0		G = 0	T = 0	T = 0	A = 0	A = 0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5.91: Genotype Data for the first 13 new CYP2C9 variants observed in the 100 Control Samples

Variants:	**Exon 1 Fragment**		**Exon 2 & 3 Fragment**							**Exon 4 Fragment**		**Exon 6 Fragment**	
Sample Number	I42V	12930 T>C	I74V	V76Q	16060 G>A	16090 T>C	16094 C>A	16179 T>A	T130T	21711 G>C	21748 G>A	46028 A>G	46092 C>T
1	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
2	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
3	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/A	G/G	G/C	G/G	A/G	C/C
4	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/A	G/G	G/C	G/G	A/G	C/C
5	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
6	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
7	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
8	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
9	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/A	C/C
10	A/A	T/T	A/A	T/A	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
11	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/T
12	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
13	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
14	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/T

15	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
16	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
17	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
18	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
19	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/T
20	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
21	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
22	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
23	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
24	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
25	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
26	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
27	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
28	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/A	G/G	G/C	G/G	A/G	C/C
29	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
30	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
31	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
32	A/A	T/T	A/A	T/T	G/G	T/T	C/C	A/A	G/G	C/C	G/G	A/G	C/C
33	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
34	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
35	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
36	A/A	T/T	A/A	T/T	G/G	T/T	C/A	T/T	G/T	G/G	G/G	A/G	C/C
37	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
38	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/A	C/C
39	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
40	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
41	A/A	T/T	A/A	T/T	G/A	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
42	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
43	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
44	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	C/C	G/G	A/A	C/C
45	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
46	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
47	A/A	C/C	A/A	T/T	G/G	C/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C

48	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
49	A/A	C/C	A/A	T/T	G/G	C/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
50	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
51	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
52	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/C	G/G	A/G	C/C
53	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
54	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
55	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/A	C/C
56	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/A	A/A	C/C
57	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
58	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
59	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
60	A/A	T/T	A/A	T/T	G/G	T/T	C/A	T/A	G/T	G/C	G/G	G/G	C/C
61	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
62	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
63	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
64	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/A	C/C
65	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
66	A/A	T/T	A/A	T/A	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
67	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
68	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
69	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
70	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
71	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
72	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
73	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
74	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/A	G/G	G/G	G/G	A/G	C/C
75	A/A	C/C	A/A	T/T	G/G	C/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
76	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
77	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
78	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
79	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
80	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C

81	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
82	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
83	A/A	T/T	A/A	T/A	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
84	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
85	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
86	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/C	G/G	A/A	C/C
87	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	G/G	C/C
88	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
89	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
90	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/A	G/G	G/C	G/G	A/G	C/C
91	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
92	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/C	G/G	A/A	C/C
93	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
94	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/C	G/G	A/A	C/T
95	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
96	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/A	C/C
97	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	G/G	C/C
98	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
99	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/G	C/C
100	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/G	C/C
TOTALS	A = 200	T = 175	A = 200	T = 197	G = 199	T = 170	C = 198	T = 171	G = 198	G = 177	G = 199	A = 154	C = 196
	G = 0	C = 25	G = 0	A = 3	A = 1	C = 30	A = 2	A = 29	T = 2	C = 23	A = 1	G = 46	T = 4
	0	19	0	3	1	24	2	27	2	19	1	40	4
	0	3	0	0	0	3	0	1	0	2	0	3	0

The variants in this table are highlighted according to the fragments in which the variants were observed. Heterozygotes are highlighted in orange and homozygotes in green.

Table 5.92: Genotype Data for the new CYP2C9 variants observed in the Exon 7, 8 and 9 fragments in the 100 Control Samples

Variants:	Exon 7 Fragment	Exon 8 Fragment					Exon 9 Fragment							
Sample Number	I327T	60175 A>G	60225 T>A	60272 T>C	60318 C>T	60328 A>T	A441A	D463D	G465G	63092 C>T	63113 C>T	63143 C>G	63169 G>A	63180 C>T
1	T/T	A/A	T/T	T/T	C/T	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
2	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
3	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
4	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
5	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
6	T/T	A/A	T/T	T/C	C/C	A/T	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
7	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
8	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
9	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
10	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
11	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
12	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
13	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
14	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
15	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
16	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
17	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/T	C/C	C/C	C/C	G/G	C/C
18	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
19	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
20	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
21	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
22	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
23	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
24	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
25	T/T	A/A	T/T	T/T	C/T	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
26	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C

27	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
28	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
29	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
30	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
31	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
32	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
33	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
34	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
35	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
36	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/T	A/A	C/C	C/C	C/C	G/G	C/C
37	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
38	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
39	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
40	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
41	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
42	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
43	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
44	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
45	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
46	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
47	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
48	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
49	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
50	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
51	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
52	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
53	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
54	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
55	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
56	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
57	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
58	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
59	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C

60	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/T	C/C	C/C	C/C	G/G	C/C
61	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/T	C/C	G/G	C/C
62	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
63	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
64	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
65	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
66	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
67	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
68	T/T	A/A	T/T	T/T	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
69	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
70	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
71	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
72	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
73	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
74	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
75	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
76	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
77	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
78	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
79	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
80	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
81	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
82	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
83	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
84	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
85	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
86	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
87	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
88	T/T	A/A	T/T	T/T	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
89	T/T	A/A	T/T	T/C	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
90	T/T	A/A	T/T	T/T	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
91	T/T	A/A	T/T	T/C	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/G	G/G	C/C
92	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C

93	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
94	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
95	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
96	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
97	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
98	T/T	A/A	T/T	T/C	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
99	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
100	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
TOTALS	T = 200	A = 200	T = 200	T = 186	C = 198	A = 199	C = 185	C = 199	A = 198	C = 200	C = 183	C = 199	G = 200	C = 200
	C = 0	G = 0	A = 0	C = 14	T = 2	T = 1	T = 15	T = 1	T = 2	T = 0	T = 17	G = 1	A = 0	T = 0
	0	0	0	14	2	1	15	1	2	0	17	1	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The variants in this table are highlighted according to the fragments in which the variants were observed. Heterozygotes are highlighted in orange and homozygotes in green.

Table 5.93: Genotype Data for the first 15 previously described CYP2C9 variants observed in the 113 Patient Samples

Sample Number	2 - R144C	3 - I354K	4 - I359T	5 - D360E	6 - 818 delA	7 - L19I	8 - R150H	9 - H251R	10 - E272G	11 - R355W	12 - P489S	13 - L90P	14 - R125H	15 - S162X
1	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
2	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
3	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
4	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
5	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
6	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
7	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
8	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
9	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
10	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C

11	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
12	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
13	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
14	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
15	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
16	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
17	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
18	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
19	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
20	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
21	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
22	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
23	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
24	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/T	C/C	T/T	G/G	C/C
25	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
26	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
27	C/C	A/A	T/T	C/C	A/A	C/C	A/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
28	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
29	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
30	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
31	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
32	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
33	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
34	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/T	C/C	T/T	G/G	C/C
35	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
36	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
37	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
38	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
39	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
40	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C

41	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
42	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
43	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
44	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
45	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
46	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
47	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
48	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
49	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
50	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
51	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
52	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
53	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
54	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
55	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
56	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
57	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
58	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
59	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
60	C/C	A/A	T/T	C/C	A/A	C/C	G/G	G/G	A/A	C/C	C/C	T/T	G/G	C/C
61	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/T	C/C	T/T	G/G	C/C
62	C/C	A/A	T/T	C/C	A/A	C/C	G/G	G/G	A/A	C/C	C/C	T/T	G/G	C/C
63	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
64	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
65	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
66	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
67	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
68	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
69	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
70	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C

71	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
72	C/C	A/A	T/T	C/C	A/A	C/C	A/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
73	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
74	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
75	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
76	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
77	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
78	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
79	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
80	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
81	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
82	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
83	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
84	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
85	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
86	C/C	A/C	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
87	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
88	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
89	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
90	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
91	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
92	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
93	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
94	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
95	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
96	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
97	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
98	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
99	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
100	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C

101	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
102	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/C	C/C	T/T	G/G	C/C
103	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/A	A/A	C/C	C/C	T/T	G/G	C/C
104	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/G	A/A	C/T	C/C	T/T	G/G	C/C
105	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
106	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
107	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
108	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
109	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
110	C/C	A/A	T/T	C/C	A/A	C/C	G/A	A/G	A/A	C/C	C/C	T/T	G/G	C/C
111	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
112	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/T	C/C	T/T	G/G	C/C
113	C/C	A/A	T/T	C/C	A/A	C/C	G/G	A/A	A/A	C/C	C/C	T/T	G/G	C/C
Totals	C = 226	A = 225	T = 226	C = 226	A = 226	C = 226	G = 196	A = 186	A = 226	C = 218	C = 226	T = 226	G = 226	C = 226
	T = 0	C = 1	C = 0	G = 0		A = 0	A = 30	G = 40	G = 0	T = 8	T = 0	C = 0	A = 0	A = 0
	0	1	0	0	0	0	26	36	0	8	0	0	0	0
	0	0	0	0	0	0	2	2	0	0	0	0	0	0

In this table the variants that have been highlighted in blue are the variants for which some patients or controls were heterozygous or homozygous and were used in the analyses described in sections three to four. Heterozygotes are highlighted in orange and homozygotes in green.

Table 5.94: Genotype Data for the previously described CYP2C9 variants 16 – 30, observed in the 113 Patient Samples

Sample Number	16 - T299A	17 - P382S	18 - D397A	19 - Q454H	20 - G70R	21 - P30L	22 - N41D	23 - V76M	24 - E354K	25 - 353-362del	26 - T130R	27 - R150L	28 - Q214L	29 - P279T	30 - A477T
1	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
2	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
3	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
4	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
5	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G

6	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
7	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
8	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
9	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
10	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
11	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
12	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
13	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
14	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
15	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
16	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
17	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
18	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
19	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
20	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
21	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
22	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
23	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
24	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
25	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
26	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
27	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	A/A	A/A	C/C	G/G
28	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
29	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
30	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
31	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
32	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
33	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
34	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
35	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G

36	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
37	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
38	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
39	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
40	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
41	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
42	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
43	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
44	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
45	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
46	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
47	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
48	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
49	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
50	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
51	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
52	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
53	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
54	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
55	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
56	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
57	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
58	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
59	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
60	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
61	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
62	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
63	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
64	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
65	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G

66	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
67	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
68	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
69	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
70	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
71	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
72	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	A/A	A/A	C/C	G/G
73	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
74	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
75	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
76	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
77	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
78	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
79	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
80	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
81	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
82	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
83	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
84	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
85	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
86	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
87	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
88	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
89	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
90	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
91	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
92	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
93	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
94	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
95	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G

96	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
97	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
98	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
99	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
100	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
101	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
102	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
103	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
104	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
105	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
106	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
107	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
108	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
109	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
110	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/A	A/A	C/C	G/G
111	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
112	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
113	A/A	C/C	A/A	G/G	G/G	C/C	A/A	G/G	G/G	No del	C/C	G/G	A/A	C/C	G/G
Totals	A = 226	C = 226	A = 226	G = 226	G = 226	C = 226	A = 226	G = 226	G = 226	Normal = 226	C = 226	G = 196	A = 226	C = 226	G = 226
	G = 0	T = 0	C = 0	C = 0	C = 0	T = 0	G = 0	A = 0	A = 0	Del = 0	G = 0	T = 0	T = 0	A = 0	A = 0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5.95: Genotype Data for the first 13 new CYP2C9 variants observed in the 113 Patient Samples

Variants:	Exon 1 Fragment		Exon 2 & 3 Fragment							Exon 4 Fragment		Exon 6 Fragment	
Sample Number	I42V	12930 T>C	I74V	V76Q	16060 G>A	16090 T>C	16094 C>A	16179 T>A	T130	21711 G>C	21748 G>A	46028 A>G	46092 C>T
1	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
2	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
3	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
4	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
5	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
6	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
7	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
8	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
9	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
10	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/T
11	A/A	T/T	A/A	T/T	G/G	T/T	C/C	A/A	G/G	C/C	G/G	G/G	C/C
12	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
13	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
14	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
15	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
16	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
17	A/A	T/C	A/A	T/T	G/G	C/C	C/C	T/A	G/G	G/C	G/G	A/G	C/C
18	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
19	A/A	T/T	A/A	T/T	G/G	T/T	C/C	A/A	G/G	C/C	G/G	G/G	C/C
20	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
21	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
22	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
23	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
24	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
25	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
26	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/G	C/C

27	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
28	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
29	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/A	G/G	G/C	G/G	A/G	C/C
30	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/A	G/G	C/C
31	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
32	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
33	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
34	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
35	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
36	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
37	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	G/G	C/C
38	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
39	A/A	C/C	A/A	T/T	G/G	C/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
40	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/A	G/G	G/C	G/G	A/G	C/C
41	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/G	C/C
42	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
43	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/T
44	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
45	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
46	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
47	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
48	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	G/G	C/C
49	A/G	T/T	A/G	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
50	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
51	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
52	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	G/G	C/C
53	A/A	T/T	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
54	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
55	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
56	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
57	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C

58	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
59	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
60	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
61	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
62	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
63	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
64	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
65	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/G	C/C
66	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
67	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/G	C/C
68	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
69	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
70	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
71	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
72	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	G/G	C/C
73	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
74	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/C	G/G	G/G	C/C
75	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/A	A/A	C/C
76	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
77	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
78	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
79	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	G/G	C/C
80	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
81	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/C	G/G	A/A	C/C
82	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/T
83	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
84	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/G	C/C
85	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/C	G/G	A/A	C/C
86	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
87	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
88	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C

89	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
90	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/C	G/G	A/A	C/C
91	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/G	G/G	A/A	C/C
92	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
93	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
94	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
95	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
96	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
97	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
98	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
99	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
100	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	G/G	C/C
101	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
102	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
103	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	G/G	C/C
104	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
105	A/A	T/T	A/A	T/T	G/G	T/T	C/A	T/A	G/T	G/C	G/G	G/G	C/C
106	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/C
107	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/A	G/G	G/C	G/G	A/G	C/C
108	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
109	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
110	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/G	C/C
111	A/A	T/T	A/A	T/T	G/G	T/T	C/C	A/A	G/G	C/C	G/G	G/G	C/C
112	A/A	T/T	A/A	T/T	G/G	T/T	C/C	T/T	G/G	G/G	G/G	A/A	C/C
113	A/A	T/C	A/A	T/T	G/G	T/C	C/C	T/T	G/G	G/G	G/G	A/A	C/T
Totals	A = 225	T = 199	A = 225	T = 226	G = 226	T = 195	C = 225	T = 198	G = 225	G = 198	G = 224	A = 161	C = 222
	G = 1	C = 27	G = 1	A = 0	A = 0	C = 31	A = 1	A = 28	T = 1	C = 28	A = 2	G = 65	T = 4
	1	25	1	0	0	27	1	22	1	22	2	39	4
	0	1	0	0	0	2	0	3	0	3	0	13	0

The variants in this table are highlighted according to the fragments in which the variants were observed. Heterozygotes are highlighted in orange and homozygotes in green.

Table 5.96: Genotype Data for the new CYP2C9 variants observed in the Exon 7, 8 and 9 fragments of the 113 Patient Samples

Variants:	Exon 7 Fragment	Exon 8 Fragment					Exon 9 Fragment							
Sample Number	I327T	60175 A>G	60225 T>A	60272 T>C	60318 C>T	60328 A>T	A441A	D463D	G465G	63092 C>T	63113 C>T	63143 C>G	63169 G>A	63180 C>T
1	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
2	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
3	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
4	T/T	A/A	T/T	T/T	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
5	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
6	T/T	A/A	T/T	T/T	C/T	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
7	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
8	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
9	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
10	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
11	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
12	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
13	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
14	T/T	A/A	T/T	T/T	C/T	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
15	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/T	C/C	C/C	C/C	G/A	C/C
16	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
17	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
18	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
19	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
20	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
21	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
22	T/T	A/A	T/T	T/T	C/C	A/T	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
23	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
24	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C

25	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
26	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
27	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	T/T	C/C	G/G	C/C
28	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
29	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
30	T/T	A/A	A/T	T/T	C/C	A/A	C/C	C/C	A/T	C/C	C/C	C/C	G/A	C/C
31	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
32	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
33	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
34	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
35	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/T	C/C	G/G	C/C
36	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
37	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/T	C/C	C/C	G/G	C/T
38	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
39	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
40	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
41	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
42	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
43	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
44	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
45	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
46	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
47	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
48	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
49	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
50	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
51	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
52	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
53	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
54	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C

55	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
56	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
57	T/T	A/A	T/T	T/C	C/T	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
58	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
59	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
60	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
61	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
62	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
63	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
64	T/C	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
65	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
66	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
67	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
68	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
69	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
70	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
71	T/T	A/A	T/T	T/T	C/C	A/T	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
72	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	T/T	C/C	G/G	C/C
73	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
74	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	T/T	C/C	G/G	C/C
75	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
76	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
77	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
78	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
79	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
80	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
81	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
82	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
83	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
84	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C

85	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
86	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/T	C/C	C/C	C/C	G/G	C/C
87	T/T	A/G	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
88	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
89	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
90	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
91	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
92	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
93	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
94	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
95	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/T	C/C	G/G	C/C
96	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
97	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
98	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
99	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
100	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
101	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
102	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
103	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
104	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
105	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/T	A/A	C/C	C/C	C/C	G/G	C/C
106	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
107	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
108	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
109	T/T	A/A	T/T	T/C	C/C	A/A	C/T	C/C	A/A	C/C	C/C	C/C	G/G	C/C
110	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/T	C/C	G/G	C/C
111	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
112	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C
113	T/T	A/A	T/T	T/T	C/C	A/A	C/C	C/C	A/A	C/C	C/C	C/C	G/G	C/C

Totals	T = 225	A = 225	T = 225	T = 212	C = 223	A = 224	C = 211	C = 225	A = 223	C = 225	C = 198	C = 226	G = 224	C = 225
	C = 1	G = 1	A = 1	C = 14	T = 3	T = 2	T = 15	T = 1	T = 3	T = 1	T = 28	G = 0	A = 2	T = 1
	1	1	1	14	3	2	15	1	3	1	22	0	2	1
	0	0	0	0	0	0	0	0	0	0	3	0	0	0

The variants in this table are highlighted according to the fragments in which the variants were observed. Heterozygotes are highlighted in orange and homozygotes in green.

Table 5.96: Genotype Data for the previously described VKORC1 variants observed in the 100 Control Samples

Sample Number	V29L	D38Y	C43C	V45A	R56G	V66M	R98W	L120L	L128R	R151G	3730 G>A
1	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
2	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
3	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
4	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
5	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
6	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
7	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
8	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
9	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
10	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
11	G/G	G/G	C/C	T/T	A/A	G/A	C/C	C/C	T/T	G/G	G/G
12	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
13	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
14	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/A
15	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
16	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
17	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
18	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
19	G/G	G/G	C/C	T/T	A/A		C/C	T/T	T/T	G/G	A/A
20	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
21	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G

22	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
23	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
24	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
25	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
26	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
27	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
28	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
29	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
30	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
31	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
32	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
33	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
34	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
35	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/A
36	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
37	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
38	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
39	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
40	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
41	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
42	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
43	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
44	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
45	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
46	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
47	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
48	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
49	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
50	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
51	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
52	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
53	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G

54	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
55	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
56	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
57	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
58	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
59	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/A
60	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
61	G/G	G/G	C/C	T/T	A/A		C/C	T/T	T/T	G/G	A/A
62	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
63	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
64	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
65	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
66	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
67	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
68	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
69	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
70	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
71	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
72	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
73	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
74	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
75	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
76	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
77	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
78	G/G	G/G	C/C	T/T	A/A						
79	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/G
80	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
81	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
82	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/A
83	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
84	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
85	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G

86	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
87	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
88	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/A
89	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/A
90	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
91	G/G	G/G	C/C	T/T	A/A		C/C	T/T	T/T	G/G	A/A
92	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
93	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
94	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
95	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
96	G/G	G/G	C/C	T/T	A/A			C/C	T/T	G/G	A/G
97	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/G
98	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
99	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	A/A
100	G/G	G/G	C/C	T/T	A/A		C/C	C/C	T/T	G/G	G/G
TOTALS	G = 200	G = 200	C = 200	T = 200	A = 200	G = 35	C = 196	C = 159	T = 198	G = 198	G = 113
	T = 0	T = 0	T = 0	C = 0	G = 0	A = 1	T = 0	T = 39	G = 0	A = 0	A = 85
	0	0	0	0	0	1	0	33	0	0	43
	0	0	0	0	0	0	0	3	0	0	21

In this table the variants that have been highlighted in blue are the variants for which some patients or controls were heterozygous or homozygous and were used in the analyses described in sections three to four. Heterozygotes are highlighted in orange and homozygotes in green. The gaps shown this table represent missing data. This was because I was unable to amplify exon 2 in the DNA of 82 of the control samples (described in section 2.2.2.2) and the sequences for 2 of the control samples for exon 3 did not cover all of the SNPs.

Table 5.97: Genotype Data for the previously described VKORC1 variants observed in the 113 Patient Samples

Sample Number	V29L	D38Y	C43C	V45A	R56G	V66M	R98W	L120L	L128R	R151G	3730 G>A
1	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
2	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
3	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
4	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G

5	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
6	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
7	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
8	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
9	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
10	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
11	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
12	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
13	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
14	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
15	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
16	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
17	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
18	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
19	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
20	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
21	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
22	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
23	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
24	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/A
25	G/G	G/G	C/C	T/T	A/A		C/C	C/T	T/T	G/G	A/A
26	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
27	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
28	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
29	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
30	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
31	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
32	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
33	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/A
34	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G

35	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
36	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
37	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
38	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
39	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
40	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
41	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
42	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
43	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
44	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
45	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
46	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
47	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
48	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
49	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
50	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
51	G/G	G/G	C/C	T/T	A/A	A/G	C/C	C/T	T/T	G/G	A/G
52	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
53	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/A
54	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
55	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
56	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
57	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
58	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
59	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
60	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
61	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
62	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
63	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
64	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G

65	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
66	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
67	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
68	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
69	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
70	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
71	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
72	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
73	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
74	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
75	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
76	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
77	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
78	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
79	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
80	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
81	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
82	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
83	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
84	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
85	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
86	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
87	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
88	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
89	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/A
90	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
91	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
92	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
93	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
94	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G

95	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
96	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
97	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
98	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
99	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
100	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
101	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/G
102	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
103	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/T	T/T	G/G	A/A
104	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
105	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
106	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
107	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
108	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
109	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
110	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
111	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	A/G
112	G/G	G/G	C/C	T/T	A/A	G/G	C/C	T/T	T/T	G/G	A/A
113	G/G	G/G	C/C	T/T	A/A	G/G	C/C	C/C	T/T	G/G	G/G
TOTALS	G = 226	G = 226	C = 226	T = 226	A = 226	G = 219	C = 226	C = 168	T = 226	G = 226	G = 127
	T = 0	T = 0	T = 0	C = 0	G = 0	A = 1	T = 0	T = 58	G = 0	A = 0	A = 99
	0	0	0	0	0	1	0	42	0	0	53
	0	0	0	0	0	0	0	8	0	0	23

In this table the variants that have been highlighted in blue are the variants for which some patients or controls were heterozygous or homozygous and were used in the analyses described in sections three to four. Heterozygotes are highlighted in orange and homozygotes in green. The gaps shown this table represent missing data. This was because I was unable to amplify exon 2 in the DNA of 3 of the patient samples (described in section 2.2.2.2) .

Clinical Data

Table 5.98: Clinical Information collected from the 113 Patient Samples

Sample Number	Age[1]	Reason on Warfarin	Dose/week[1]	INR[1]
1	36	Mitral valve repair	27.5	2.4
2	40	Artificial valve	52.5	3.39
3	38	Artificial valve	70	1.41
4	44	Mitral valve repair	35	1.52
5	28	Artificial valve	35	2.89
6	30	Artificial valve	60	1.43
7	31	Artificial valve	52.5	2.38
8	39	Mitral valve repair	40	4.3
9	36	Artificial valve	62.5	2.35
10	30	Artificial valve	45	2.43
11	28	Artificial valve	35	2.18
12	26	Artificial valve	52.5	1.74
13	38	Artificial valve	35	1.75
14	43	Artificial valve	40	2.4
15	31	Artificial valve	42.5	2.41
16	33	Artificial valve	47.5	2.88
17	26	Artificial valve	35	2.31
18	44	Artificial valve	40	1.79
19	31	Artificial valve	77.5	1.78
20	33	Artificial valve	52.5	1.05
21	44	Mitral Stenosis	35	3.83
22	30	Artificial valve	75	3.51
23	38	Artificial valve	22.5	1.58
24	26	Artificial valve	35	6.17
25	38	Artificial valve	35	2.39
26	30	Artificial valve	35	3.47
27	44	Artificial valve	52.5	3.39

28	37	Artificial valve	47.5	1.15
29	24	Artificial valve	42.5	1.44
30	43	Artificial valve	40	4.68
31	38	Artificial valve	47.5	1.45
32	30	Artificial valve	52.5	2.52
33	45	Artificial valve	17.5	1.99
34	40	Artificial valve	42.5	1.31
35	31	Artificial valve	65	2
36	31	Mitral valve repair	35	2.55
37	27	Artificial valve	17.5	3.21
38	35	Artificial valve	42.5	1.24
39	23	Artificial valve	45	1.69
40	26	Artificial valve	87.5	6.56
41	28	Artificial valve	47.5	2.35
42	39	Artificial valve	35	1.58
43	45	Artificial valve	35	2.77
44	43	Artificial valve	37.5	2.71
45	42	Artificial valve	42.5	1.11
46	35	Artificial valve	65	1.81
47	35	Artificial valve	65	4.07
48	36	Artificial valve	25	2.21
49	41	Artificial valve		2.43
50	31	Artificial valve	42.5	3.82
51				
52	36	Artificial valve	42.5	2.3
53	37	Artificial valve	52.5	1.2
54	37	Artificial valve	42.5	2.27
55	34	Artificial valve	30	3.44
56	29	Artificial valve	35	4.09
57	31	Artificial valve	87.5	1.07
58	45	Artificial valve	47.5	1.74
59	29	Artificial valve	42.5	0.96

60	41	Artificial valve	55	3.31
61	37	Artificial valve	35	5.39
62	39	Artificial valve	62.5	4.95
63	44	Artificial valve	35	3.51
64	27	Artificial valve	35	1.08
65	39	Artificial valve	12.5	2.25
66	45	Artificial valve	42.5	1.84
67	27	Artificial valve	47.5	2.41
68	38	Artificial valve	35	1.48
69	36	Artificial valve	35	2.65
70	34	Artificial valve	35	2.76
71	34	Artificial valve	35	2.08
72	37	Artificial valve	35	2.7
73	34	Artificial valve	35	1.34
74	42	Artificial valve	52.5	1.04
75	24	Artificial valve	42.5	1.8
76	30	Artificial valve	40	1.09
77	42	Artificial valve	42.5	2.89
78	27	Artificial valve	40	2.68
79	39	Artificial valve	25	1.34
80	35	Artificial valve	25	1.08
81	30	Post-surgery for AV canal defect	35	1.11
82	36	Artificial valve	42.5	2.74
83	28	Artificial valve	60	2.23
84	32	Artificial valve	40	1.57
85	42	Artificial valve	52.5	5.51
86	30	Artificial valve	30	3.05
87	28	Artificial valve	42.5	2.02
88	36	Artificial valve	17.5	5.01
89	33	Artificial valve	35	1.44
90	43	Artificial valve	52.5	1.09
91	33	Artificial valve	35	1.56

92	41	Artificial valve	35	0.96
93	35	Mitral valve repair	42.5	1.03
94	37	Artificial valve	60	3.63
95	38	Artificial valve	27.5	2.22
96	34	Artificial valve	35	3.55
97	29	Mitral valve repair	52.5	2.17
98	26	Artificial valve	47.5	1.15
99	39	Artificial valve	35	1.47
100	29	Artificial valve	35	2.67
101	43	Artificial valve	35	1.46
102	34	Pulmonary Hypertension	45	5.35
103	44	Artificial valve	25	2.14
104	28	Artificial valve	25	3.3
105	36	Artificial valve	22.5	3.24
106	44	Artificial valve	60	1.5
107	37	Artificial valve	42.5	2.89
108	29	Artificial valve	30	1.33
109	33	Artificial valve	35	2.3
110	37	Artificial valve	25	2.58
111				
112	38	Artificial valve	27.5	2.15
113	31	Artificial valve	45	1.62

[1] Age, Dosage and INR taken at the time of the study

Table 5.99: Concomitant Medication information collected from the 113 Patient Samples

Sample Number	Digoxin	Lasix	Slow K	Beta-block	Aspirin	Aldactone	Moduretic	ACE - I	Nifedipine	Cordarone	Isoptin	Amiloride	Epanutin	Tegretol	Amitrypt
1	yes	no	no	yes	no	no	yes	no	no	no	no	no	no	no	no
2	no	yes	yes	no	yes	no	no	no	no	no	no	no	no	no	no
3	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
4	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
5	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
6	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
7	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
8	yes	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
9	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
10	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
11	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
12	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
13	no	yes	yes	no	no	no	yes	no	no	no	no	no	no	no	no
14	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
15	no	yes	yes	no	no	no	no	yes	no	no	no	no	no	no	no
16	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
17	no	yes	yes	no	yes	no	no	no	no	no	no	no	no	no	no
18	no	yes	yes	no	yes	no	no	no	no	no	no	no	no	no	no
19	no	no	no	no	yes	no	no	no	no	no	no	no	no	yes	no
20	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
21	no	yes	yes	yes	no	yes	no	no	no	no	no	no	no	no	no
22	yes	yes	yes	no	no	no	no	yes	no	no	no	no	no	no	no
23	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
24	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
25	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
26	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
27	yes	yes	yes	no	yes	no	no	yes	no	no	no	no	no	no	no
28	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no

29	no	no	no	no	no	no	yes	no	no	no	no	no	no	no	no
30	no	no	no	no	no	no	yes	no	no	no	no	no	no	no	no
31	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
32	no	no	no	yes	yes	no	yes	no	no	no	no	no	no	no	no
33	yes	yes	yes	no	no	yes	no	yes	no	no	no	no	no	no	no
34	no	yes	yes	no	yes	yes	no	no	no	no	no	no	no	no	no
35	no	yes	yes	no	yes	no	yes	no	no	no	no	no	no	no	no
36	yes	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
37	no	yes	yes	no	yes	no	no	no	no	no	no	no	no	no	no
38	no	no	no	no	no	no	no	yes	no	no	no	no	no	no	no
39	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
40	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
41	no	no	no	no	no	no	no	yes	no	no	no	no	no	no	no
42	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
43	yes	yes	yes	no	no	no	no	yes	no	yes	no	no	no	no	no
44	no	yes	yes	no	no	yes	no	no	no	no	no	no	no	no	no
45	no	yes	yes	no	no	yes	no	no	no	no	no	no	no	no	no
46	no	yes	yes	no	no	no	no	yes	no	no	no	no	no	no	no
47	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
48	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
49	yes	yes	yes	no	yes	no	no	no	no	no	no	no	no	no	no
50	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
51	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
52	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
53	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
54	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
55	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
56	no	yes	yes	yes	no	no	no	no	no	yes	no	no	no	no	no
57	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
58	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
59	no	no	yes	no	yes	no	yes	no	no	no	no	no	no	no	no
60	no	no	no	no	no	no	yes	no	no	no	no	no	no	no	no

61	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
62	yes	no	no	no	no	no	yes	no	no	no	yes	no	no	no	no
63	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
64	no	yes	no	no	yes	no	no	yes	no	no	no	no	no	no	no
65	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
66	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
67	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
68	yes	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
69	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
70	no	no	no	no	no	no	no	yes	no	no	no	no	no	no	no
71	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
72	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
73	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
74	yes	yes	yes	no	no	no	no	no	no	yes	no	no	yes	no	no
75	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
76	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
77	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
78	no	no	no	yes	no	yes	no	no	no	no	no	no	no	no	no
79	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
80	no	no	no	yes	no	no	yes	yes	no	no	no	no	no	no	no
81	no	yes	no	no	no	yes	no	yes	no	yes	no	no	no	no	no
82	no	yes	yes	no	yes	no	no	no	no	no	no	no	no	no	no
83	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
84	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
85	no	no	no	no	no	no	no	no	no	yes	no	no	no	no	no
86	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
87	yes	yes	no	no	yes	no	no	no	no	no	no	no	no	no	no
88	no	yes	yes	no	no	no	no	no	no	yes	yes	no	no	no	no
89	no	no	no	yes	yes	no	no	no	no	no	no	no	no	no	no
90	yes	no	no	yes	no	yes	no	yes	no	no	no	no	no	no	no
91	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
92	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

93	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
94	yes	yes	yes	no	yes	no	no	yes	no	no	no	no	no	no	no
95	no	no	no	no	yes	no	no	no	no	no	no	yes	no	no	yes
96	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
97	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
98	no	no	no	no	no	no	no	no	yes	no	no	no	no	no	no
99	yes	yes	yes	yes	yes	no	no	no	no	yes	no	no	no	no	no
100	no	no	no	no	yes	no	yes	no	no	no	no	no	no	no	no
101	yes	yes	yes	no	yes	no	no	no	no	no	no	no	no	no	no
102	no	yes	no	no	no	no	no	no	yes	no	no	no	no	no	no
103	yes	yes	yes	no	no	no	no	yes	no	no	no	no	no	no	no
104	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
105	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
106	no	yes	no	no	yes	no	no	yes	no	no	no	no	no	no	no
107	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no
108	no	no	no	no	yes	no	yes	no	no	no	no	no	no	no	no
109	no	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
110	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
111	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
112	yes	yes	yes	yes	no	no	no	no	no	no	no	no	no	no	no
113	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no

Table 5.100: Pregnancy information collected from the 113 Patient Samples

Sample Number	Total Number of Preg	Preg 1 Outcome	Preg 2 Outcome	Preg 3 Outcome	Preg 4 Outcome	Preg 5 Outcome	Preg 6 Outcome	Preg 7 Outcome
1	3	miscarriage	normal liveborn	miscarriage				
2	4	normal liveborn	normal liveborn	stillbirth	TOP			
3	1	stillbirth						
4	6	normal liveborn	normal liveborn	normal liveborn	normal liveborn, baby died	normal liveborn	normal liveborn	
5	3	normal liveborn	miscarriage	stillbirth				
6	1	normal liveborn, baby died						
7	1	Stillbirth						
8	3	normal liveborn	normal liveborn	Physically & mentally abnormal				
9	3	abnormal liveborn, mentally abnormal	stillbirth	Stillbirth, physically abnormal				
10	1	normal liveborn						
11	1	normal liveborn						
12	1	miscarriage						
13	3	normal liveborn	normal liveborn	stillbirth				
14	3	miscarriage	Physically & mentally abnormal	normal liveborn				
15	3	normal liveborn	normal liveborn	miscarriage				
16	4	stillbirth	stillbirth	normal liveborn	stillbirth			
17	1	miscarriage						
18	6	normal liveborn	miscarriage	normal liveborn	normal liveborn	normal liveborn	stillbirth	
19	2	miscarriage	miscarriage					

20	5	normal liveborn	normal liveborn	normal liveborn	miscarriage	miscarriage		
21	3	normal liveborn	normal liveborn	normal liveborn				
22	1	stillbirth						
23	2	normal liveborn	normal liveborn					
24	2	Physically abnormal liveborn	normal liveborn					
25	3	normal liveborn	miscarriage	miscarriage				
26	1	normal liveborn, baby died						
27	4	normal liveborn	Physically & mentally abnormal	normal liveborn	normal liveborn			
28	3	stillbirth	normal liveborn	normal liveborn				
29	2	miscarriage	stillbirth					
30	5	normal liveborn	normal liveborn	normal liveborn	normal liveborn	abnormal liveborn, baby died		
31	2	normal liveborn	stillbirth					
32	2	miscarriage	miscarriage					
33	3	normal liveborn	normal liveborn	Physically abnormal				
34	5	normal liveborn	stillbirth	normal liveborn	normal liveborn	TOP		
35	3	normal liveborn	normal liveborn	miscarriage				
36	5	stillbirth	stillbirth	stillbirth	normal liveborn	TOP		
37	3	stillbirth	stillbirth	normal liveborn				
38	2	normal liveborn	physical, mental abnormal					
39	1	stillbirth						
40	1	stillbirth						

41	2	normal liveborn	normal liveborn					
42	5	normal liveborn	normal liveborn	stillbirth	stillbirth	stillbirth		
43	3	normal liveborn	normal liveborn	abnormal liveborn, baby died				
44	5	normal liveborn	normal liveborn	normal liveborn	normal liveborn	normal liveborn		
45	3	normal liveborn	normal liveborn	stillbirth				
46	4	stillbirth	normal liveborn	miscarriage	normal liveborn			
47	1	ectopic						
48	1	normal liveborn						
49	1	normal liveborn						
50	2	miscarriage	normal liveborn					
52	2	normal liveborn	normal liveborn					
53	2	normal liveborn, baby died	miscarriage					
54	4	normal liveborn	stillbirth	normal liveborn	Physically abnormal			
55	1	miscarriage						
56	1	normal liveborn						
57	1	normal liveborn						
58	3	normal liveborn	miscarriage	miscarriage				
59	2	normal liveborn	normal liveborn					
60	3	stillbirth	stillbirth	normal liveborn				
61	3	normal liveborn	normal liveborn	stillbirth				
62	4	stillbirth	miscarriage	normal liveborn, baby died	stillbirth			
63	2	miscarriage	stillbirth					
64	2	normal liveborn	ectopic					
65	3	normal liveborn	normal liveborn	miscarriage				
66	4	normal liveborn	normal liveborn	normal liveborn	mental abnormal			

67	2	stillbirth	normal liveborn					
68	1	abnormal liveborn, baby died						
69	4	normal liveborn	stillbirth	normal liveborn	stillbirth			
70	3	miscarriage	miscarriage	miscarriage				
71	2	stillbirth	miscarriage					
72	7	normal liveborn	normal liveborn	miscarriage	ectopic	miscarriage	miscarriage	miscarriage
73	1	Physically abnormal liveborn						
74	4	miscarriage	normal liveborn	stillbirth	stillbirth			
75	1	normal liveborn						
76	3	abnormal liveborn, baby died	miscarriage	stillbirth				
77	5	normal liveborn	miscarriage	miscarriage	normal liveborn, baby died	normal liveborn		
78	1	normal liveborn						
79	1	Physically abnormal liveborn						
80	2	normal liveborn	physically abnormal					
81	3	miscarriage	normal liveborn	miscarriage				
82	3	normal liveborn	normal liveborn	normal liveborn				
83	2	normal liveborn	normal liveborn					
84	1	stillbirth						
85	2	normal liveborn	normal liveborn					
86	1	normal liveborn						
87	1	normal liveborn						
88	1	normal liveborn						

89	1	abnormal liveborn, baby died						
90	8	normal liveborn	normal liveborn	stillbirth	stillbirth	stillbirth	stillbirth	stillbirth
91	2	stillbirth	stillbirth					
92	1	ectopic						
93	5	normal liveborn	normal liveborn	normal liveborn	stillbirth	miscarriage		
94	2	miscarriage	miscarriage					
95	4	normal liveborn	normal liveborn	normal liveborn	normal liveborn			
96	2	normal liveborn	normal liveborn					
97	2	normal liveborn	ectopic					
98	1	miscarriage						
99	5	normal liveborn	miscarriage	TOP	stillbirth	stillbirth		
100	2	normal liveborn	miscarriage					
101	2	normal liveborn	normal liveborn					
102	4	normal liveborn	normal liveborn	miscarriage	miscarriage			
103	3	normal liveborn	normal liveborn	Physically abnormal				
104	3	miscarriage	normal liveborn	normal liveborn				
105	2	normal liveborn	normal liveborn					
106	2	normal liveborn	normal liveborn					
107	2	normal liveborn	normal liveborn					
108	1	normal liveborn						
109	2	normal liveborn	Mentally abnormal					
110	6	normal liveborn	stillbirth	stillbirth	miscarriage	miscarriage	stillbirth	
112	2	normal liveborn	normal liveborn					
113	1	normal liveborn						

Appendix N

This appendix contains pictures of some agarose gels and electropherograms used in this project, referred to in the first paragraph of page 44.

Agarose Gel Pictures

Figure 5.3 shows an agarose gel picture of PCR amplicons of exon 6 of the *CYP2C9* gene in 46 control samples. Figure 5.4 shows an agarose gel picture of PCR amplicons of exon 8 of the *CYP2C9* gene of 46 patient samples. Figure 5.5 shows an agarose gel picture of PCR amplicons of exon 3 of the *VKORC1* gene in 46 control samples

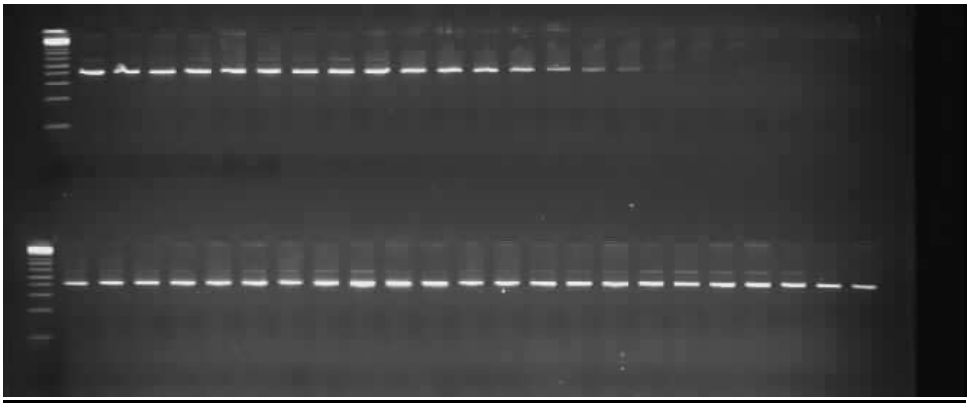


Figure 5.3: Picture of an agarose gel run with *CYP2C9* exon 6 fragments

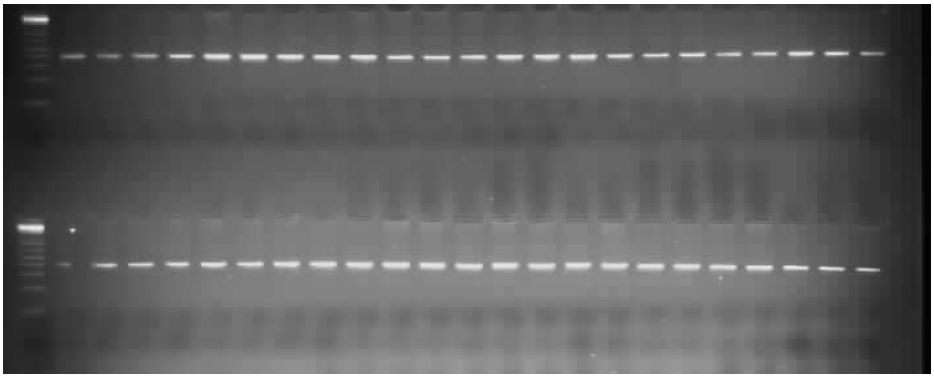


Figure 5.4: Picture of an agarose gel run with *CYP2C9* exon 8 fragments

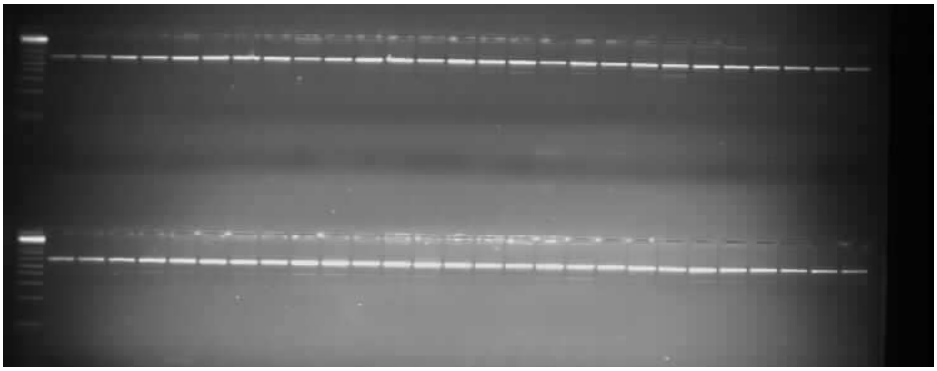


Figure 5.5: Picture of an agarose gel run with *VKORC1* exon 3 fragments

Electropherograms

The following pictures are electropherograms taken from the 3130xl genetic analyzer for exon 1 of the *VKORC1* gene for control sample 1 and exon 3 of the *CYP2C9* gene for patient sample 4, respectively.

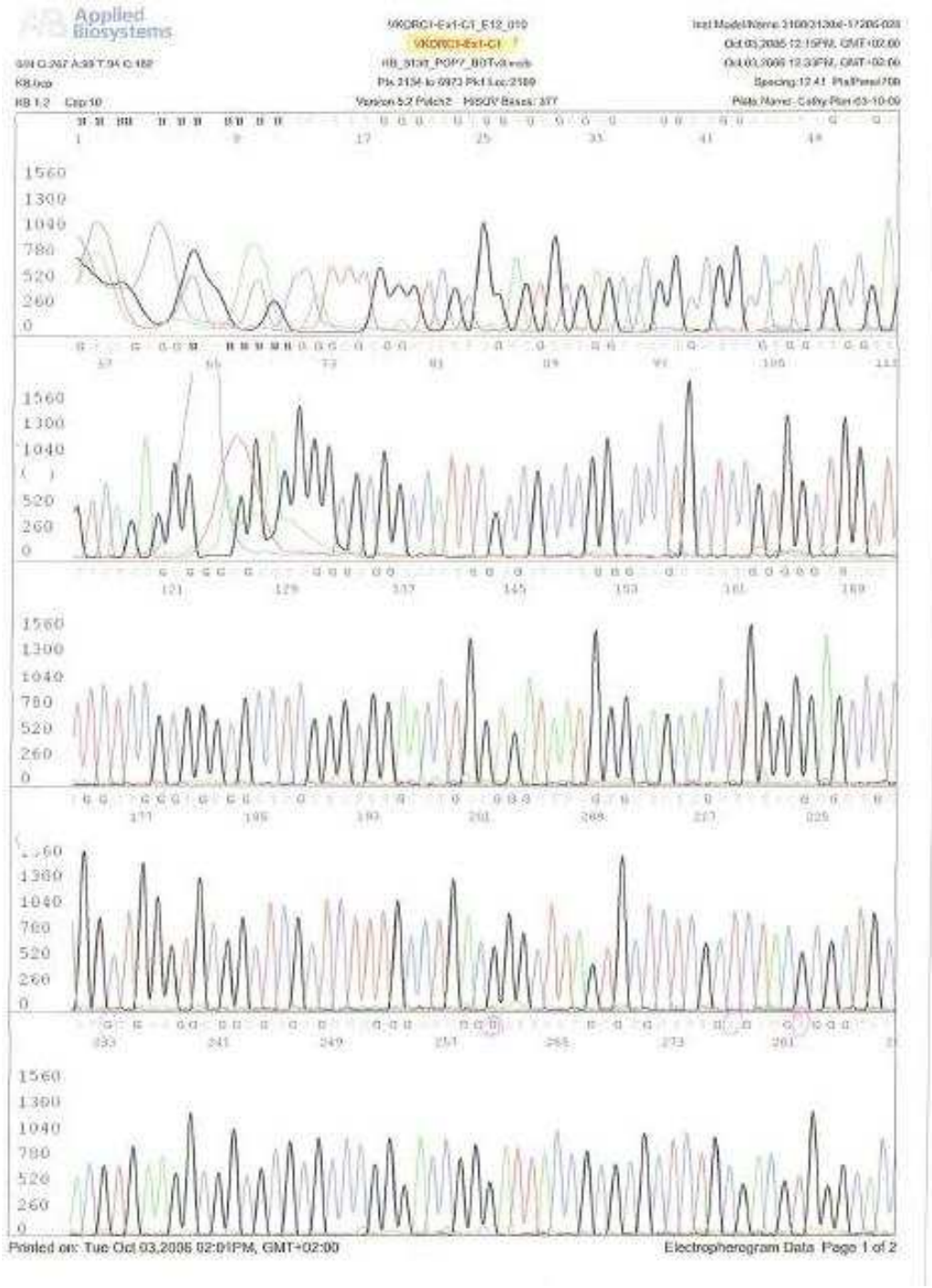


Figure 5.6: Electropherogram of *VKORC1* exon 1 in control sample 1

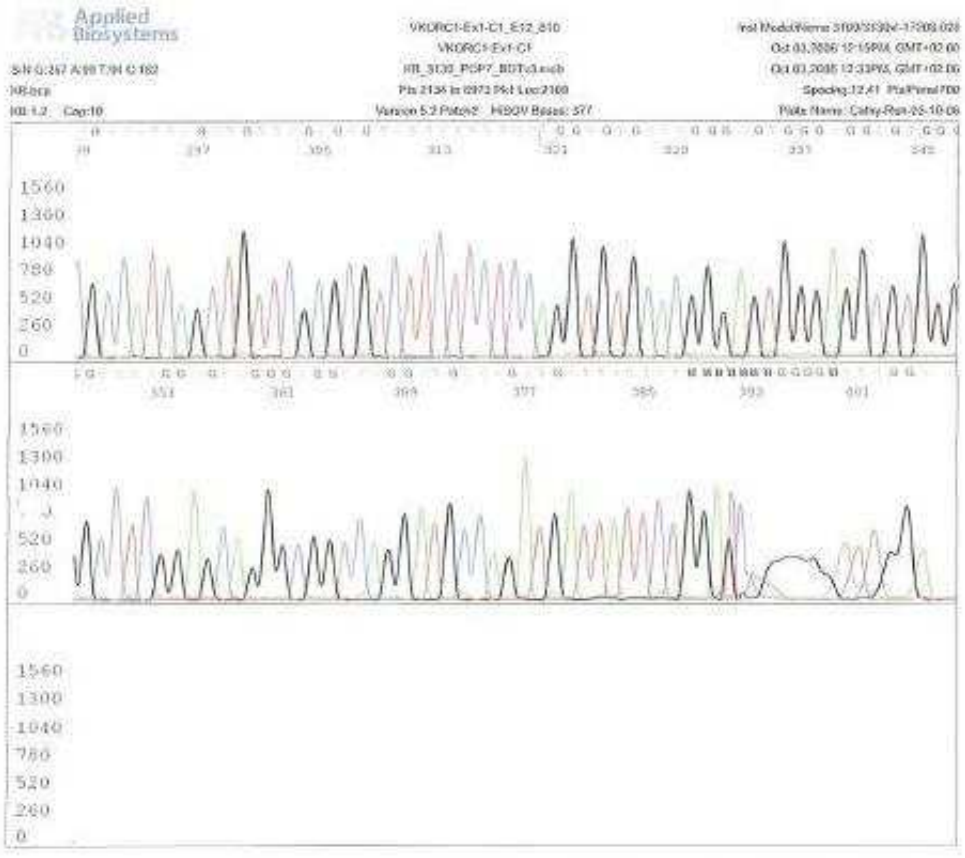


Figure 5.7: Electropherogram of *VKORC1* exon 1 in control sample 1, continued

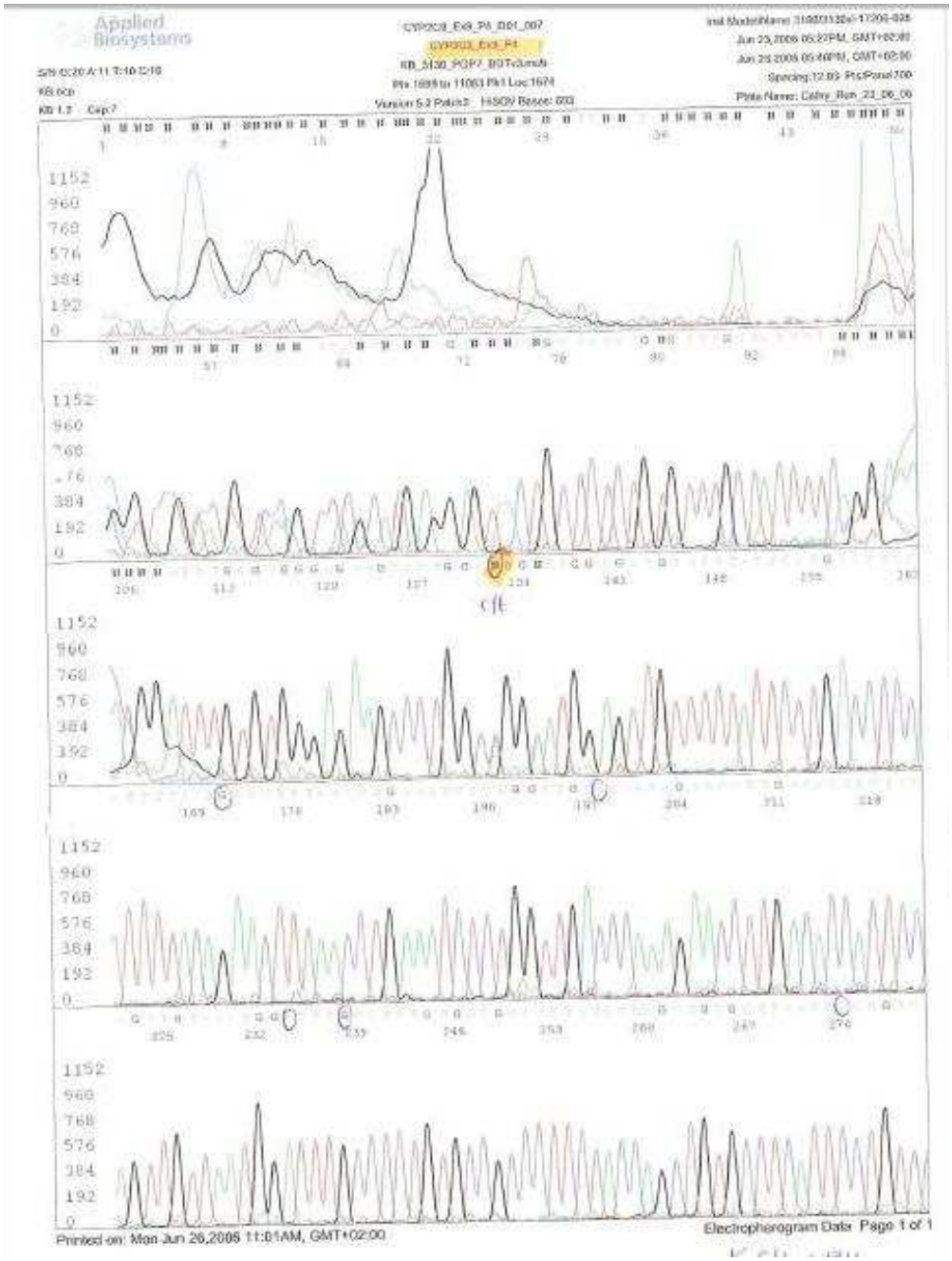


Figure 5.8: Electropherogram of *CYP2C9* exon 3, patient 4

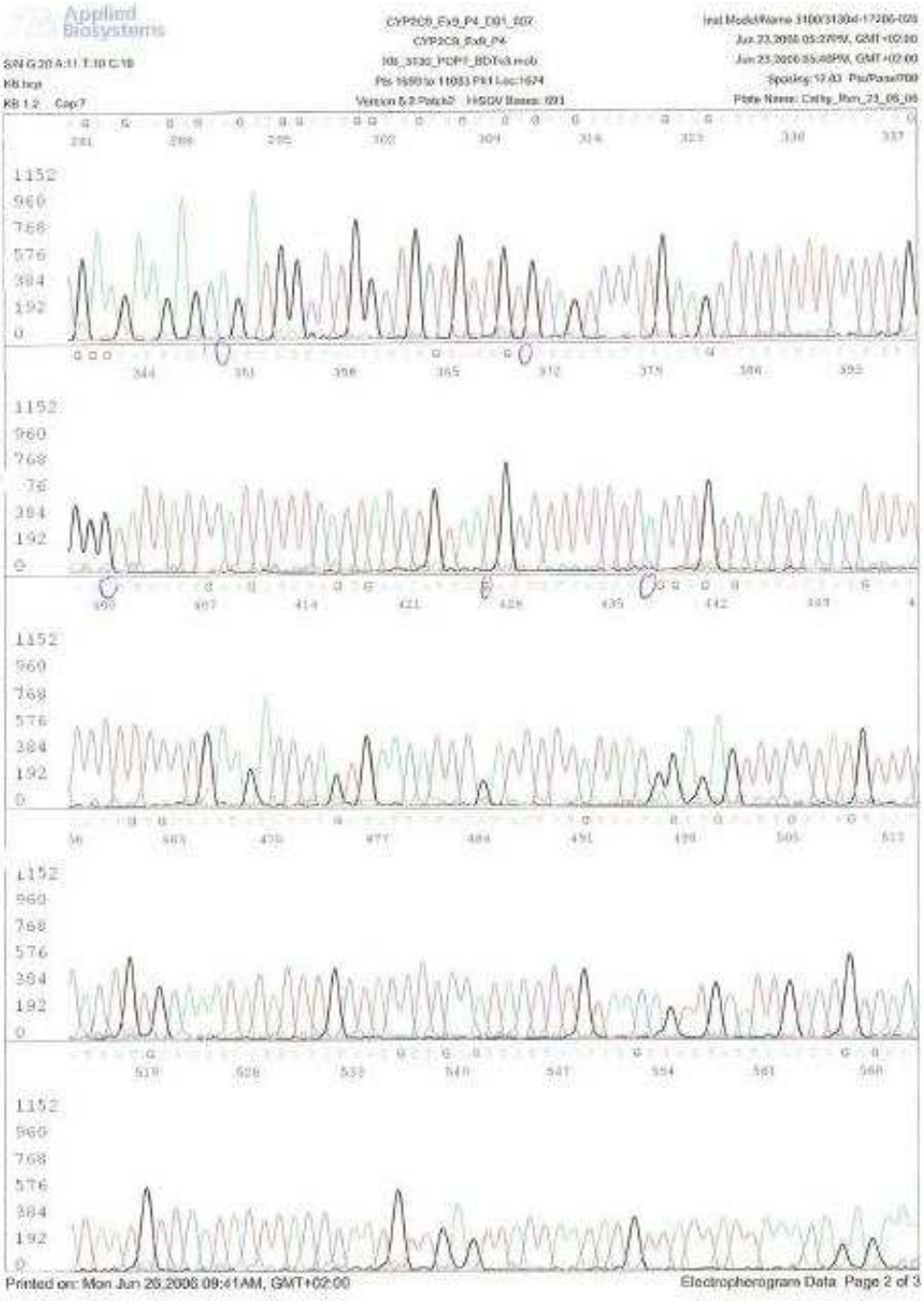


Figure 5.9: Electropherogram of *CYP2C9* exon 3, patient 4, continued

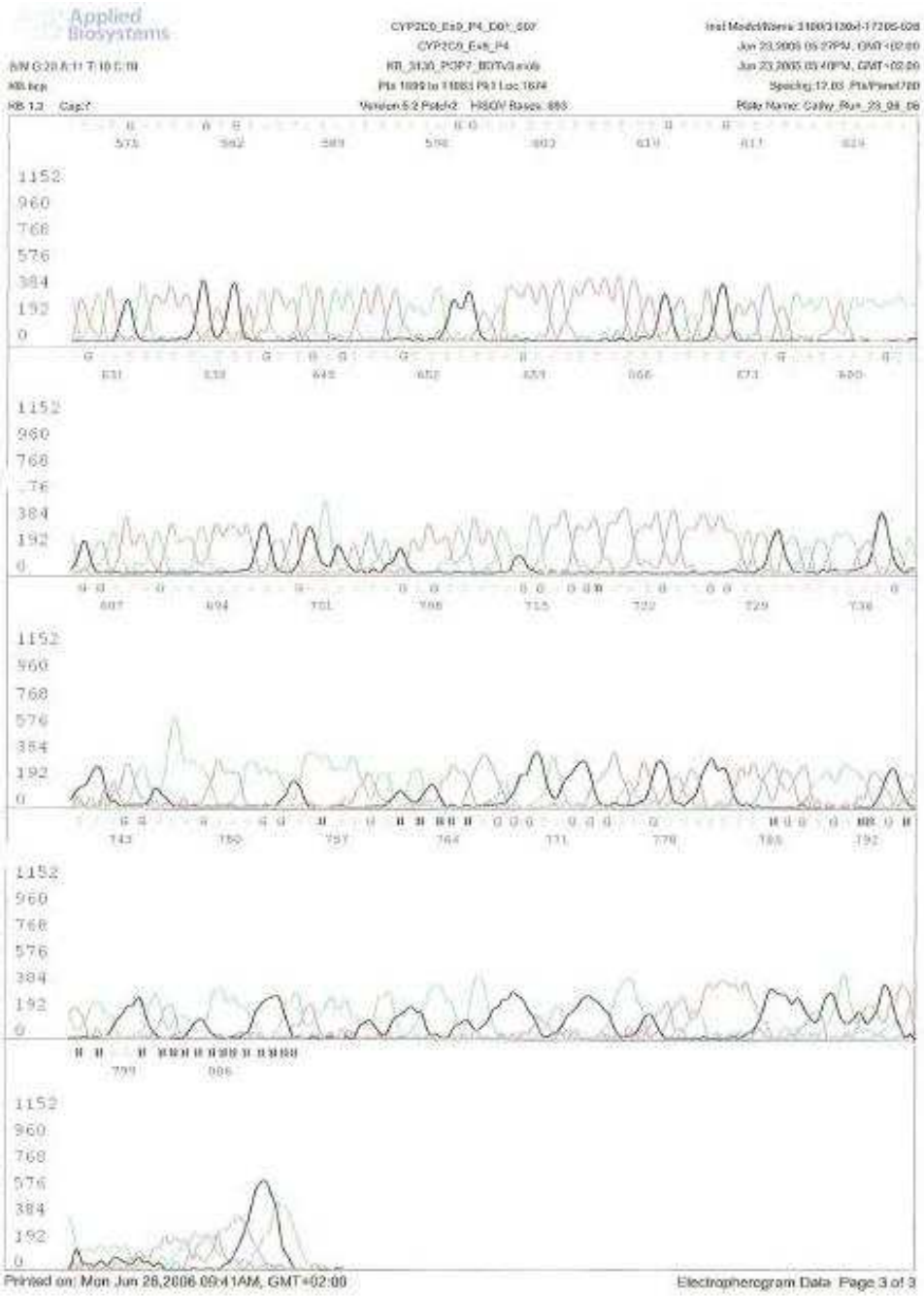


Figure 5.10: Electropherogram of *CYP2C9* exon 3, patient 4, continued

6 REFERENCES

Electronic References:

Alex Dong Li's Splice Site Finder: www.genet.sickkids.on.ca/~ali/splicesitefinder.html

Berkeley Drosophila Genome Project Splice Site Predictor: www.fruitfly.org/seq_tools/splice.html

CYP2C9 allele nomenclature: www.cypalleles.ki.se/cp2c9.htm

Encyclopaedia Britannica: Pygmy. http://www.britannica.com/eb/article-9062017/Pygmy

Ensembl. http://www.ensembl.org/Homo_sapiens/geneview?gene=ENSG00000167397

Frett R (2007) Effect of advanced age on fertility and pregnancy in women. Up to date patient information. http://patients.uptodate.com/topic.asp?file=antenatl/14857

GenBank: www.ncbi.nlm.nig.gov

Heart Health: iVillage Total Health. http://heart.health.ivillage.com

Kimball Genetics Website: www.kimballgenetics.com

Merriam-Webster's Medical Dictionary: www.m-w.com/medical

Millipore: www.millipore.com and www.millipore.com/montage

National Centre for Biotechnology Information (NCBI) database: www.ncbi.nih.gov

NetDoctor: http://www.netdoctor.co.uk/medicines

NetGene2 Server: www.cbs.dtu.dk/services/NetGene2

Next Generation Pharmaceutical: Pharmacogenetics and warfarin therapy. www.ngpharma.com/pastissue/article.asp?art=269086&issue=185

OMIM (Online Mendelian Inheritance in Man): www.ncbi.nlm.nig.gov

The African American MS Genetics Project: http://www.ucsf.edu/msdb/newsletter/2005/2005AA.pdf

The Royal Society (2005) Personalised medicines: hopes and realities: http://royalsociety.org/document.asp?id=3780

RX List: The internet drug index. www.rxlist.com

UCSC Genome Bioinformatics Database Website: http://genome.ucsc.edu

Software References:

Clayton D (2006) dgc.genetics: Additions/enhancements of the genetics package. R package version 0.5

Clayton D (Unknown) Practical exercise on population - based case/control studies. R package version 0.5.

Hastie T, Tibshirani R (2007) GAM: Generalized additive models. Department of statistical software - Standford University: stat.standford.edu

Lasergene V5, SeqMan Pro™. DNASTAR Inc, Madison, WI, USA

R Development Core Team (2006) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3 -900051-07-0, URL http://www.R-project.org

Shin J, Blay S, Lewin-Koh N, et al., (unknown) LDheatmap: Graphical display of pairwise linkage disequilibria between SNPs. R package version 0.2-3. http://stat-db.stat.sfu.ca:8080/statgen/research/LDheatmap/

Sinnwell JP, Schaid DJ (2005) haplo.stats: Statistical Analysis of Haplotypes with Traits and Covariates when Linkage Phase is Ambiguous. R package version 1.2.2. http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth edition. Springer, New York. ISBN 0-387-95457-0

Warnes G, Leisch F (2005) Genetics: Population Genetics. R Package Version 1.2.0.

Literature References:

Aithal GP, Day CP, Kesteven PJL, et al., (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with Warfarin dose requirements and risk of bleeding complications. The Lancet 353: 717-719

Allabi AC, Gala J, Desager J, et al., (2003) Genetic polymorphisms of *CYP2C9* and *CYP2C19* in Beninese and Belgian populations. British Journal of Clinical Pharmacology 56: 653-657

Allabi AC, Gala J, Horsmans Y, et al., (2004) Functional impact of CYP2C9*5, CYP2C9*6, CYP2C9*8 and CYP2C9*11 in vivo among black Africans. Clinical Pharmacology and Therapeutics 76: 113-118

Bamshad M (2005) Genetic influences on health. Journal of the American Medical Association 294: 937-946

Bajoria R, Sooranna SR, Contractor SF (1996) Differential binding of Warfarin to maternal, foetal and non-pregnant sera and its clinical implications. Journal of Pharmacy and Pharmacology 48: 486-491

Blaisdell J, Jorge-Nebert LF, Coulter S, et al., (2004) Discovery of new potentially defective alleles of human CYP2C9. Pharmacogenetics 14: 527-537

Brenner B, Sanchez-Vega B, Wu SM (1998) A missense mutation in gamma-glutamyl carboxylase gene causes combined deficiency of all vitamin K-dependent blood coagulation factors. Blood 92: 4554-4559

Cain D, Hutson SM, Wallin R (1997) Assembly of the warfarin-sensitive vitamin K 2,3-epoxide reductase enzyme complex in the endoplasmic reticulum membrane. Journal of Biological Chemistry 272: 29068-29075

Chan WS, Anand S, Ginsberg JS (2000) Anticoagulation of pregnant women with mechanical heart valves. Archives of Internal Medicine 160: 191-196

Chan YC, Valenti D, Mansfield AO et al., (2000) Warfarin induced skin necrosis. British Journal of Surgery 87: 266-272

Cotrufo M, De Feo M, De Santo LS et al., (2002) Risk of Warfarin during pregnancy with mechanical valve prosthesis. Journal of Obstetrics and Gynaecology 99:35-40

D'Andrea G, D'Ambrosia RL, Di Perna P, et al., (2005) A polymorphism in the *VKORC1* gene is associated with an interindividual variability in the dose-anticoagulant effect of Warfarin. Blood 105 (2): 645-649

Dahlback B (2005) Blood coagulation and its regulation by anticoagulant pathways: genetic pathogenesis of bleeding and thrombotic diseases. Journal of Internal Medicine 257: 209-223

Dickmann LJ, Rettie AE, Kneller MB, et al., (2001) Identification and functional characterization of a new CYP2 variant (CYP2C9*5) expressed among African Americans. Molecular Pharmacology 60: 382-387

Ensom MH, Chang TK, Patel P (2001) Pharmacogenetics: the therapeutic drug monitoring of the future? Clinical Pharmaco-kinetics 40: 783-802

Ginsberg JS, Greer I, Hirsh J (2001) Use of antithrombotic agents during pregnancy. Chest 119: 122S-131S

Gray IC, Nobile C, Muresu R, et al., (1995) A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. Genomics 28: 328-332

Greaves M (2005) Pharmacogenetics in the management of coumarin anticoagulant therapy: the way forward or an expensive diversion? Public library of Science: Medicine 2 (10): 944-945

Gregersen NE (2005) The implications to women of childbearing age taking Warfarin anticoagulation [Dissertation] Johannesburg South Africa: University of the Witwatersrand

Hall JG, Pauli RM, Wilson KM (1980) Maternal and fetal sequelae of anticoagulation during pregnancy. The American Journal of Medicine 68: 122-137

Hall DR, Olivier J, Rossouw GJ et al., (2001) Pregnancy outcomes in women with prosthetic heart valves. Journal of Obstetrics and Gynaecology 21: 149-153

Harrington DJ, Underwood S, Morse C et al., (2005) Pharmacodynamic resistance to warfarin associated with a Val66Met substitution in vitamin K epoxide reductase complex subunit 1. Journal of Thrombosis and Haemostasis 93: 23-26

Hillman MA, Wilke RA, Caldwell MD, et al., (2004) Relative impact of covariates in prescribing Warfarin according to CYP2C9 genotype. Pharmacogenetics 14: 539-547

Hillman MA, Wilke RA, Yale SH, et al., (2005) A prospective, randomised pilot trial of model-based Warfarin dose initiation using CYP2C9 genotype and clinical data. Clinical Medicine and Research 3: 137-145

Hirsch J, Dalen JE, Anderson DR, et al., (1998) Oral anticoagulants: mechanism of action, clinical effectiveness and optimal therapeutic range. Chest 114: 4455-4695

Horton JD, Bushwick BM (1999) Warfarin therapy: evolving strategies in anticoagulation. American Family Physician 59 (3): 1-18

Imai K, Takahashi S, Kamahori M, et al., (1999) Multi-capillary DNA sequencer. Hitachi Review 48 (3): 107-109

Imai J, Ieiri I, Mamiya K et al., (2000) Polymorphism of the cytochrome P450 (CYP) 2C9 gene in Japanese epileptic patients: genetic analysis of the *CYP2C9* locus. Pharmacogenetics 10: 85-89

Kalow W (2005) Pharmacogenetics and pharmacogenomics: origin, status, and the hope for personalised medicine. The Pharmacogenomics Journal 6: 162-165

Kresge N, Simoni RD and Hill RL (2005) Haemorrhagic sweet clover disease, dicumarol and warfarin: The work of Karl Paul Link. Journal of Biological Chemistry 280: e5-e6

Li T, Chang C, Jin D, et al., (2004) Identification of the gene for vitamin K epoxide reductase. Nature 427: 541-543

McGraw-Hill (2002) McGraw-Hill Dictionary of Scientific and Technical Terms, 6th edition, published by The McGraw-Hill Companies, Inc.

Mathews CK, Van Holde KE, Ahern KG (2000) Biochemistry 3rd Edition. Addison Wesley Longman, Inc. pg 129

Miller SA, Dyk DD, Pelesky HF, et al., (1988) A Simple Salting-Out Procedure for Extracting DNA from Human Nucleated Cells. Nucleic Acid Research 16: 1215.

Morisseau C, Hammock BD (2005) Epoxide hydrolases: mechanisms inhibitor designs, and biological roles. Annual Reviews of Pharmacology and Toxicology 45: 311-333

Mueller RF, Young ID (2001) Emery's Elements of Medical Genetics 11th Edition. Churchill Livingstone. Pg 352 - 353

Nakagawa T, Kishino S, Itoh S, et al., (2003) Differential binding of disopramide and Warfarin enantiomers to human alpha(1)-acid glycoprotein variants. British Journal of Clinical Pharmacology 56: 664-669

O'Reilly RA, Aggeler PM, Leong LS (1963) Studies on the coumarin anticoagulant drugs: The pharmacodynamics of warfarin in man. Journal of Clinical Investigation 42 (10): 1542 - 1551

Reider MJ, Reiner AP, Gage BF, et al., (2005) Effect of VKORC1 haplotypes on transcriptional regulation and Warfarin dose. New England Journal of Medicine 352: 2285-2293

Releford J (2001) Global analysis of regional differences in Craniometric diversity and population substructure. Human Biology 73(5): 629-636

Resta R, Biesecker BB, Bennett RL, et al., (2006) A new definition of genetic Counselling: National Society of Genetic Counselors' Task Force Report. Journal of genetic counselling 15(2): 77-83

Rettie AE, Korzekwa KR, Kunze KL, et al., (1992) Hydroxylation of warfarin by cDNA-expressed cytochrome P450: A role for P4502C9 in the etiology of (S)-warfarin-drug-interactions. Chemical Research in Toxicology 5: 54-59

Rettie AE, Tai G (2006) The pharmacogenomics of warfarin: closing in on personalized medicine. Molecular Interventions 6(4): 223-227

Roderick LM (1931) A problem in the coagulation of the blood; "sweet clover disease of the cattle". American Journal of Physiology 96: 413-416

Rost S, Fregin A, Ivaskevicius V, et al., (2004) Mutations in *VKORC1* cause Warfarin resistance and multiple coagulation factor deficiency type 2. Nature 427: 537-540

Rost S, Fregin A, Koch D et al., (2004) Compound heterozygous mutations in the gamma-glutamyl carboxylase gene cause combined deficiency of all vitamin K-dependent blood coagulation factors. British Journal of Haematology 126: 546-549

Sadler I, McCowan L, White H et al., (2000) Pregnancy outcomes and cardiac complications in women with mechanical, bioprosthetic and homograft valves. British Journal of Obstetrics and Gynaecology 107: 245-253

Sareli P, England MJ, Berk MR et al., (2000) Pregnancy outcomes and cardiac complications in women with mechanical, bioprosthetic and homograft valves. American Journal of Cardiology 63: 1462-1465

Serre D, Paabo S (2004) Evidence for gradients of human genetic diversity within and among continents. Genome Research 14: 1679-1685

Schwarz UI, Richie MD, Bradford Y, et al., (2008) Genetic determinants of response to warfarin during initial anticoagulation. New England Journal of Medicine 358(10): 999-1008

Schofield FW (1924) Damaged sweet clover; the cause of a new disease in cattle simulating haemorrhagic septicaemia and blackleg. Journal of the American Veterinary Medical Association 64: 553-556

Shapiro SS (2003) Treating Thrombosis in the 21st Century. New England Journal of Medicine 349: 1762-1764

Si D, Guo Y, Zhang Y et al., (2004) Identification of a novel variant *CYP2C9* allele in Chinese. Pharmacogenetics 14: 465-469

Stahmann MA, Huebner CF, Link KP (1941) Studies on the hemorrhagic sweet clover disease. V. Identification and synthesis of the hemorrhagic agent. Journal of Biological Chemistry 138: 513-527

Strachan T, Read PR (2004) Human molecular genetics 3rd Edition. Garland Science, Taylor and Francis group, New York and London.

Tishkoff SA, Kidd KK (2004) Implications of biogeography of human populations for "race" and medicine. Nature Genetics 36(11): S21-S27

Wadelius M, Pirmohammed M (2007) Pharmacogenetics of Warfarin: current status and future challenges. The Pharmacogenomics Journal 7(2): 99-111

Wadelius M, Chen LY, Eriksson N, et al., (2007) Association of warfarin dose with genes involved in its action and metabolism. Human Genetics 121: 23-34

Wilson K, Walker J (2000) Practical biochemistry: principles and techniques 5th Edition. Cambridge University Press, Cambridge, UK

Wolf CR, Smith G, Smith RL (2000) Science, medicine and the future: Pharmacogenetics. The British Medical Journal 320:987-990

Yin T, Miyata T (2007) Warfarin dose and pharmacogenomics of *CYP2C9* and *VKORC1* – rationale and perspectives. Thrombosis Research 120: 1-10

Zhao F, Loke C, Rankin SC, et al., (2004) Novel CYP2C9 genetic variants in Asian subjects and their influence on maintenance Warfarin dose. Clinical Pharmacology and Therapeutics 76 (3): 210-219