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Association of Genetic Polymorphisms in Vitamin K Epoxide
Reductase and GAS6 haplotypes with Recurrent Pregnancy Loss
among Palestinian Women

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and GAS6 haplotypes with Recurrent Pregnancy Loss among Palestinian
Women

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I certify that this thesis submitted for the degree Master of Science is the result of my own research, except where otherwise acknowledged, and that this thesis (or part of the same) has not been submitted for a higher degree to any other University or Institution

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Date

Dedication

To those who taught me the beauty of life
& the joy of science.....

Mom and Dad

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Abstract

Understanding the relationship of Single Nucleotide Polymorphisms (SNPs) with the incidence of disease is a step towards individualized medicine. The main objective of this study is to explore the potential association of SNPs in Vitamin K epoxide reductase complex subunit 1 (*VKORC1*) gene and Growth Arrest- Specific 6 (*GAS6*) gene with the occurrence of unexplained Recurrent Pregnancy Loss (RPL) and Breast Cancer.

Variants in *VKORC1* gene have been found to affect the amount of reduced vitamin K (VK), a cofactor for γ -carboxylation of vitamin K-dependent proteins. The C allele of the *VKORC1* +2255 T/C SNP is associated with higher activity at this locus and has been linked to increased vascular events. VK is important for post-modification of clotting factors involved in the coagulation cascade and Vitamin K₂ (VK₂) exerts an apoptotic effect on cancer cells. Studying a polymorphism which affects the status of VK in the body may be significant for a thrombophilia approach to unexplained RPL and Breast Cancer incidence.

Gas6 (Growth Arrest- Specific 6) is a Vitamin K dependent protein that exerts an anti-apoptotic effect by interacting with receptor tyrosine kinase, TAM family; Tyro3, Axl and MerTK. *GAS6* expression has been found to be up-regulated in several types of cancer; this protein is also involved in clot stability. The A allele of the *GAS6* polymorphism c.834+7G>A may have a protective role against thrombophilia. This allele is possibly linked with a decrease in expression. The *GAS6* c.834+7G>A SNP was explored for a potential protective role in cancer and RPL.

The same SNPs were screened in both Breast Cancer and RPL cases, but were considered for different prospects and each was approached as a separate case- control type study. Genotyping was performed by using *Nco*I and *Alw*N I restriction enzymes for *VKORC1* and *GAS6* SNPs, respectively.

In the RPL study, 45 patients and 77 age matched controls were screened at the loci in question, no significant difference in haplotype distribution was observed for either *GAS6* c.834+7G>A ($P = 0.83$) or *VKORC1* +2255 T/C ($P = 0.20$) among the groups. This polymorphism maybe thrombophilia unrelated in the unexplained RPL cases. Further research is recommended to explore the significance of these SNPs in the Palestinian population.

In the Breast Cancer study, 81 patients and 84 controls were analysed for the indicative haplotypes. No significant difference was observed in the allele distribution for the *GAS6* c.834+7G>A SNP among all participants ($P = 0.32$). However, a significant difference in haplotype distribution for the *VKORC1* +2255T/C SNP was observed ($P = 0.02$). The TT haplotype was found in 32% of the Breast Cancer patients, and in only 16.7% of the control group. The CC and CT haplotypes were found in 83.3% of controls and 67.9% of patients. The T allele conferred a more than 2 fold increased risk for developing Breast Cancer OR 2.36, 95% CI (1.13 - 4.95).

Further work is needed to explain the association of the T allele of the *VKORC1* gene with Breast Cancer and other factors which may affect VK status in the body.

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Index of Abbreviations

A	Adenine
BC	Breast Cancer
C	Cytosine
CI	Confidence Interval
df	degrees of freedom
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphates
G	Guanine
Gla	γ -carboxyglutamic acid
Kb	Kilo base pairs
OR	Odds Ratio
<i>P</i>	Probability
<i>P</i> ₁	Primer 1
<i>P</i> ₂	Primer2
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
mRNA	Messenger ribonucleic acid
RPL	Recurrent Pregnancy Loss
RTKs	receptor tyrosine kinases
SNP	Single Nucleotide Polymorphisms
T	Thymine
TAE	Tris-acetate-EDTA
VK ₁	Vitamin K ₁
VK ₂	Vitamin K ₂
χ^2 -test	Chi- Square Test

Chapter I

Introduction

Single nucleotide polymorphisms (SNPs) are the most common genetic variations among humans; it is a change in a single nucleotide at any location in the genome. Studying the pattern among SNPs in specific targeted genes and the incidence of a particular disease or response to a medical treatment, is one of the first steps towards a more individualized and prophylactic approach to medicine. Case-control SNP association studies are increasingly popular, populations may differ drastically in haplotype frequencies, therefore the findings of one study does not necessarily apply to all. It is important for research in this area to be conducted in the Palestinian population, in order to help transform our current health system to a more personalized approach. This thesis will explore the potential association between SNPs in the *VKORC1* and *GAS6* genes with the occurrences of Breast Cancer and Recurrent Pregnancy Loss (RPL). The background information for the rationale of this study is provided in the sections of this chapter.

1.1.1 Vitamin K

Vitamin K is a fat-soluble vitamin discovered in the 1930s, during cholesterol experiments in chickens (Dam and Schonheyder, 1934). Vitamin K (VK) is a collective term for several related chemical compounds, they all share a 2-methyl-1, 4 naphthoquinone backbone structure, but differ in the composition of the side chain at position C-3 (Oldenburg *et al.*, 2008). Vitamin K1 (VK₁), also known as phylloquinone, is present in cyanobacteria and plants; it possesses a mostly saturated C-20 phytyl side chain. Vitamin K2 (VK₂) is produced by microbial organisms and is characterized by a partly unsaturated, predominantly C-40 side chain (menaquinone). In organisms that produce VK, both types are involved in electron transport processes (Oldenburg *et al.*, 2008).

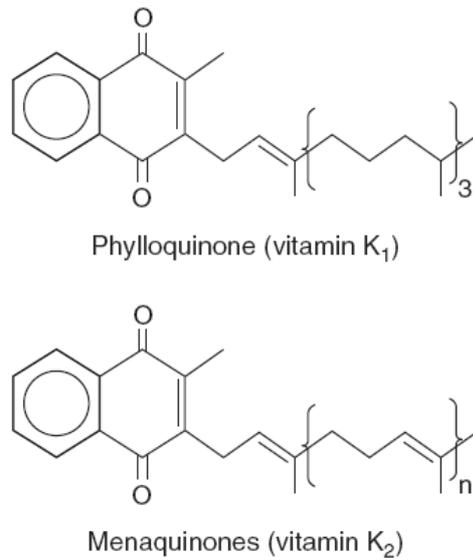


Fig 1.1 Vitamin K structures (Wallin R *et al.*, 2008)

1.1.2 Vitamin K dependent proteins

Vitamin K is an essential participant in posttranscriptional modification of proteins involved in coagulation, calcium metabolism, and other physiological processes (Oldenburg *et al.*, 2008). All of these proteins are modified by carboxylation of glutamic acid residues to form γ -carboxyglutamic acid (Gla), in the absence of vitamin K the carboxylation does not occur and the proteins are biologically inactive (Oldenburg *et al.*, 2008). VK dependent proteins in the coagulation cascade include the clotting factors II, VII, IX, X, and anticoagulant proteins C, S and Z which are integral to regulating hemostasis (Martinez and Barsigian, 1998). Each of these proteins is γ -carboxylated at several amino terminal glutamyl residues, which enables Ca^{+2} binding (Garcia and Reitsma, 2008 and Oldenburg *et al.*, 2008).

Osteocalcin and matrix Gla-protein are γ -carboxylated proteins involved in bone metabolism. Gas6 is another VK dependent protein; some of its functions include cell growth and survival. In addition, four presumed carboxylated transmembrane proteins (abbreviated as PRGP1, PRGP2, TmG3, and TmG4) are predicted to be VK dependent although their biological function remains to be identified (Kulman *et al.*, 2001 and Oldenburg *et al.*, 2006).