Genetic polymorphisms and the cardiovascular risk of nonsteroidal anti-inflammatory drugs

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Abstract

The cardiovascular safety of cyclooxygenase-2 (COX-2) selective non-steroidal anti-inflammatory drugs (NSAIDs) is of concern, although the majority of users remain free of adverse outcomes. A gene-drug interaction may contribute to the variation in individual response to NSAIDs.

In a case-only study of 460 patients selected from a cohort admitted for an acute coronary syndrome we genotyped 115 single nucleotide polymorphisms (SNPs). We observed statistically significant gene-drug interactions between NSAID exposure and 16 SNPs. Of these, four interactions strengthened and remained significant in a COX-2 subgroup including a SNP in the C-Reactive Protein (CRP) gene (OR=3.6; 95% Confidence Interval [CI], 1.6 – 7.9; P=0.001), two SNPs in the cyclooxygenase-1 (COX-1) gene (OR=6.9; 95% CI, 1.4 - 35.7; P=0.02 and OR=6.9; 95% CI, 1.3 - 35.6; P=0.02) and one SNP in the Klotho gene (OR=2.3; 95% CI, 1.4-3.9; P=0.002).

Genetic polymorphisms within the COX-1, CRP and Klotho genes are candidates for gene-drug interactions influencing the cardiovascular outcomes of users of NSAIDs. These findings suggest that genetic susceptibility may contribute to coronary instability in some users of this class of drugs.

Abrégé

L'innocuité des médicaments anti-inflammatoires non stéroïdiens (AINS) inhibiteurs sélectifs de la cyclooxygénase-2 (COX-2) pour le système cardiovasculaire soulève des préoccupations, bien que ces médicaments ne produisent aucun effet indésirable chez la majorité de leurs utilisateurs. Une interaction gène-médicament pourrait jouer un rôle dans les variations entre les réactions individuelles aux AINS.

Dans une étude de 460 cas choisis parmi une cohorte de patients admis pour un syndrome coronarien aigu, nous avons établi le génotype de 115 polymorphismes d'un nucléotide simple (PNS). Nous avons observé des interactions gène-médicament statistiquement significatives entre l'exposition aux AINS et 16 PNS. De ce nombre, quatre interactions se sont accentuées et sont demeurées significatives dans un sous-groupe recevant des coxibs incluant un PNS du gène de la Protéine C réactive (PCR) (OU=3,6; 95 % Intervalle de confiance [IC], 1,6 – 7,9; P=0,001), deux PNS du gène de la cyclooxygénase-1 (COX-1) (OU=6,9; 95 % IC, 1,4 – 35,7; P=0,02 et OU=6,9; 95 % IC, 1,3 – 35,6; P=0,02) un PNS du gène de la Klotho (OU=2,3; 95 % IC, 1,4-3,9; P=0,002).

Les polymorphismes génétiques des gènes COX-1, PCR et Klotho présentent un profil favorable aux interactions gène-médicament qui contribuent aux effets sur le système cardiovasculaire des utilisateurs d'AINS. Ces résultats indiquent que la sensibilité génétique peut favoriser l'instabilité coronarienne chez certains utilisateurs de cette classe de médicaments.

Dedication

This thesis is dedicated to my Grandpa, Charles Edward Frost (1919 - 2007), who always took the time to remind me that he had all the faith in the world in me. I love you and miss you dearly.

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Contribution of Authors

This thesis includes the text of a manuscript that will be submitted for publication. I (Christine St.Germaine) wrote the application for funding for the MUHC pilot project competition, the ethics protocol, the thesis, and the manuscript. I used the existing database and the suggested study design to develop the study methods. I also reviewed the literature and suggested a list of possible candidate genes to study. I was primarily responsible for the statistical analysis conducted in SAS

Dr. James Brophy was primarily responsible for the supervision of this project. He proposed the research question and suggested the case-only study design. He provided comments, suggestions and revisions on the application for funding to the MUHC pilot project competition, all chapters in the thesis and drafts of the manuscript.

Dr. Jamie Engert shared his knowledge and guidance in genetics. He answered questions regarding genetics databases and software used in this thesis. He contributed to the selection of candidate genes and provided advice on the methods of statistical analysis for this genetic study as well as comments, suggestions and revisions for the thesis and the manuscript.

Dr. Hanley attended committee meetings and provided statistical advice. He also offered comments and suggestions to improve the quality of the manuscript and the thesis.

Dr. Peter Bogaty provided the genetic samples and database of patient information used in this study. Luce Boyer assisted in the retrieval and delivery of genetic samples.

All authors contributed to the interpretation of the results of this study.

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Chapter 1

Introduction and Literature Review

1. Introduction

Despite the efficacy of selective cyclooxygenase-2 (COX-2) inhibitors for the treatment of osteoarthritis, menstrual pain, acute pain and rheumatoid arthritis, these drugs may be associated with an increase in adverse cardiovascular outcomes. The potent selective COX-2 inhibitor, rofecoxib (Vioxx) was voluntarily withdrawn from the worldwide market due to the results of multiple studies demonstrating increased cardiovascular risk including the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial and the Vioxx GI Outcomes Research (VIGOR) study.[1-3] Since then, another COX-2 inhibitor, valdecoxib (Bextra), has been recalled due to similar cardiovascular risks and serious skin reactions.[4] These events have raised concern that increased cardiovascular risk may be a class effect prompting further investigation into the safety of all non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors.

Although the cardiovascular events observed among users of COX-2 inhibitors should not be ignored, the absolute rate of patients experiencing a thrombotic event in the APPROVe trial was fortunately low at 1.5 per 100 patient years suggesting that the majority of patients were treated without incident.[2] The biological mechanism that triggers these cardiovascular events is still unclear; however, previous research has shown that the individual therapeutic response to COX-2 inhibitors varies and that this variation may be associated with **genetic polymorphisms*** within the COX pathway.[5-7] Therefore, it is plausible that the presence of one or more genetic polymorphisms may create an environment in which a COX-2 inhibitor will trigger cardiovascular events leading to a myocardial infarction or stroke.

This study seeks to investigate **gene**-drug interactions between candidate **single nucleotide polymorphisms (SNPs)** and the use of a NSAID or COX-2 inhibitor that may contribute to the cardiovascular risk associated with this class of drugs. The findings of this study should advance the understanding of the genetic contribution to the cardiovascular safety of COX-2 inhibitors with potential implications for the development of anti-inflammatory pharmaceuticals and genetic screening for drug safety.

* Words and phrases in bold are defined in the Genetic Epidemiology Glossary in Appendix 1

1.1 A brief history of NSAIDs and selective cyclooxygenase-2 (COX-2) inhibitors

As early as ancient Egypt and Greece when willow bark was used to treat joint and wound pain, humans have capitalized on the pain reducing and anti-inflammatory properties of salicylates.[8] However, it was not until the 1870's that one of the first "clinical trials" of the use of a NSAID for the treatment of pain and inflammation was documented. In his report, Dr. Thomas MacLagan used 2g of salicin derived from the common white willow to treat the fever, pain and inflammation associated with rheumatic fever.[8, 9] It was also during the 1870's that Salicylic acid was derived from salicin and its chemical structure was identified leading to industrial production of the drug.[8] Shortly thereafter, Felix Hoffmann, a chemist with Bayer and company synthesized acetylsalicylic acid and in 1899 the trade name "Aspirin" was registered.[8] Fifty years later, the first non-aspirin NSAID, ibuprofen, was identified and within the next twenty years it gained popularity as a prescription for painful musculoskeletal conditions.[8]

In 1999, a new generation of NSAIDs, known as selective COX-2 inhibitors or coxibs, were introduced to the Canadian market. This class of drugs includes rofecoxib (Vioxx), celecoxib (Celebrex), valdecoxib (Bextra) and the most recently approved, lumiracoxib (Prexige). While traditional NSAIDs target both of the main COX isoforms ranging from high selectivity towards COX-1 to equal activity on both, coxibs have a higher affinity for COX-2 than COX-1 and were designed to treat pain and inflammation without the gastrointestinal (GI) side effects associated with the inhibition of COX-1.[10] It is important to note that COX-2 binding affinity varies even among COX-2 selective agents and COX-2 selectivity is dose-dependent.[11]

1.2 The biological mechanism of NSAIDs and selective COX-2 inhibitors

Despite the early discovery and use of salicylates, the biological mechanism responsible for the therapeutic efficacy of NSAIDs was not discovered until 1971

when three reports appeared in the journal *Nature* demonstrating that NSAIDs reduce prostaglandin formation as a result of inhibition of the cyclooxygenase (COX) enzyme.[9, 12-14] Eleven years later, Sir John Vane along with Bengt Samuelsson and Sune Bergstrom received the Nobel Prize in physiology and medicine for this work.[15]

Their investigations found that the cyclooxygenase enzyme facilitates the oxidation of arachidonic acid to hydroperoxy endoperoxide prostaglandin G_2 and its further reduction to hydroxyl endoperoxide prostaglandin H2 which is then used to synthesize the primary prostanoids including prostaglandin E_2, $F_2\alpha$, D_2, I_2 and thromboxane A_2.[16] These prostanoids have many roles including maintaining the stomach mucosa, regulating platelet aggregation and mediating the inflammatory response.[17] (Figure 1.1)

To date, two main isoforms of the COX enzyme, COX-1 and COX-2, have been identified. COX-1 is encoded by the **constitutively** expressed prostaglandin-endoperoxide synthase 1 (PTGS1) **gene** and produces prostaglandins (PGs) involved in the regulation of stomach mucosa, platelet aggregation, and kidney function.[18] COX-2 is encoded by the **inducible** PTGS2 gene and is rapidly induced by inflammatory cytokines and mitogens; therefore, it is thought to be responsible for producing the majority of prostaglandins involved in inflammation and cancer.[18] However, the most important function of COX-2 is its role in mediating the production of prostaglandin I_2 (PGI_2). This prostaglandin, also known as prostacyclin, is involved in maintaining vascular homeostasis through its role in vasorelaxation and inhibiting platelet aggregation and vascular smooth muscle growth.[19] A third isoform, a variant of COX-1 known as COX-3, has recently been described in the literature but its role in relation to NSAIDs is still under investigation.[20]

Increased understanding of the different roles of COX-1 and COX-2 in the prostaglandin pathway led to the development of selective COX-2 inhibitors designed to inhibit the prostaglandins responsible for inflammation while maintaining the function of prostaglandins regulating stomach mucosa. Since 1999, several selective COX-2 inhibitors have been introduced to the Canadian

market including rofecoxib, celecoxib, valdecoxib and lumiracoxib. However, an observed increase in cardiovascular events among users of COX-2 inhibitors suggests that there may be other clinical consequences of selectively inhibiting COX-2.

1.3 Adverse effects of NSAIDs and selective COX-2 inhibitors

In general, the safety profile for non-selective non-steroidal anti-inflammatory drugs is favourable. However, NSAIDs do carry a risk of GI bleeding which can be life threatening.[21] Although clinical trials provide evidence that selective COX-2 inhibitors are associated with fewer gastrointestinal events compared with traditional non-selective NSAIDs, the cardiovascular risk profile of coxibs has been called into question.[3, 22]

1.4 COX-2 selective inhibitors and myocardial infarction: A class effect?

The first observation that coxibs may compromise cardiovascular health came in 2000 with the publication of the Vioxx (rofecoxib) Gastrointestinal Outcomes Research (VIGOR) study which found the rate of myocardial infarction to be four times greater among individuals assigned to rofecoxib compared to naproxen (0.4% vs. 0.1%, respectively).[3, 23] However, at this time it was reported that the difference in cardiovascular risk may be due to a cardioprotective effect of naproxen driven by the drug's ability to inhibit thromboxane and reduce platelet aggregation.[3, 23]

That same year, a similar large randomized controlled trial of celecoxib, known as the Celecoxib Long-term Arthritis Safety Study (CLASS), did not show any significant difference in cardiovascular events among patients treated with celecoxib, compared with ibuprofen or diclofenac.[22] However, the CLASS study allowed concomitant aspirin use and it was unclear whether this was attenuating cardiovascular effects.[22]

The results of these trials prompted further investigation into the possible cardiovascular toxicity of coxibs and three pooled analyses were published shortly thereafter.[24-26] Of these, one independent study confirmed the findings of the VIGOR study while two industry sponsored studies did not report cardiogenic effects.[24-26]

On September 30, 2004, four years after the publication of the VIGOR study, Merck announced a voluntary worldwide withdrawal of rofecoxib because cardiovascular toxicity was observed in the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial.[2] Shortly thereafter, valdecoxib (Bextra) was recalled by the United States Food and Drug Administration (FDA) due to evidence of similar cardiovascular side effects as well as severe skin reactions. Evidence of increased cardiovascular risk among celecoxib users has been less convincing. Although an early clinical trial and one observational study suggest an increase in cardiovascular events, several observational studies have shown no evidence of harm.[27-30] Despite this variation in risk, drug regulators remain cautious and black box warnings regarding cardiovascular toxicity have been placed on all non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors.[31] To date, the question remains whether cardiovascular toxicity is a class effect of NSAIDs or specific to one or more of the coxibs. Furthermore, the biological mechanism by which cardiovascular toxicity occurs and the genetic contribution to this mechanism has yet to be elucidated.

1.5 Genetic associations with NSAIDs and/or COX-2 inhibitors

The biological plausibility of a genetic contribution to the safety of COX-2 inhibitors and NSAIDs is supported by evidence that the efficacy of these drugs is modulated by genetic variation.[5, 6] Furthermore, several studies have shown that coxibs and NSAIDs are also associated with changes in the expression of genes linked to other inflammatory pathways.[5, 6, 32] Moreover, it is also plausible that NSAIDs may accelerate cardiovascular events if an individual is already genetically susceptible to cardiovascular complications. Although our study focuses on the two enzyme targets of COX-2 inhibitors and NSAIDs, COX-1 and

COX-2, we also chose to investigate several **candidate genes** and many candidate SNPs with known associations with NSAIDs, COX-2 inhibitors or cardiovascular disease processes.

1.5.1 The COX-1 and COX-2 Genes (PTGS1 and PTGS2)

NSAIDs target the two main isoforms of the cyclooxygenase enzyme, COX-1 and COX-2. The prostaglandin endoperoxide synthase 1 (PTGS1) and prostaglandin endoperoxide synthase 2 (PTGS2) genes encode these proteins, respectively. Although it is intuitive that the PTGS1 and PTGS2 genes may contain single nucleotide polymorphisms that could influence the efficacy or safety of NSAIDs or COX-2 inhibitors, recent research also supports this hypothesis. According to a study by Lee *et al*, ibuprofen and rofecoxib significantly altered the expression of PTGS2 and this expression was significantly different among individuals with a G/G **allele** at the position **-765 G>C** (rs20417) in the PTGS2 gene, compared with individuals with the G/C and C/C alleles.[5] Furthermore, the degree of pain relief experienced upon treatment with rofecoxib and ibuprofen varied by allele group.[5] These results suggest that functional polymorphisms within the PTGS2 gene may be predictive of the analgesic efficacy of NSAIDs and COX-2 inhibitors.

Moreover, Lee et al. report that, in vitro, several genetic variants in the PTGS1 gene alter arachidonic acid metabolism.[33] Furthermore, a previous study showed that **heterozygotes** at two SNPS located within the PTGS1 **locus** showed significantly greater inhibition of prostaglandin H_2 formation upon administration of aspirin compared with **homozygote** carriers of the common allele.[34] Therefore, we decided to investigate if polymorphisms within PTGS1 or PTGS2 may also influence the inter-individual variation in the safety of NSAIDs.

1.5.2 The Prostacyclin Synthase Gene (PTGIS)

It has also been hypothesized that NSAIDs and COX-2 inhibitors may reduce the production of prostacyclin also known as prostaglandin (PG) I_2.[35] PGI_2 is the most potent inhibitor of platelet aggregation and a strong vasodilator

suggesting a role as a cardioprotective mediator.[17, 36] Consequently, it is also believed to be involved in vascular remodeling diseases and to play important roles in angiogenesis and apoptosis.[36] Furthermore, prostacyclin couples with COX-2 to sustain production of prostacyclin in circulation and endothelial cells.[36] The production of PGI_2 is regulated by the Prostaglandin I_2 Synthase gene (PTGIS) which facilitates the isomerization of Prostaglandin H_2 (PGH_2) to PGI_2.[37] Therefore, we hypothesize that variants in this gene may interact with NSAIDs or COX-2 inhibitors to promote cardiovascular events.

1.5.3 The Matrix Metalloproteinase (MMP) Genes

Previous research has shown that COX-2 derived prostaglandins are involved in the regulation of the matrix metalloproteinase (MMP) genetic pathway in various cell types.[32] Recently, Wang *et al* found rofecoxib alters the expression of several genes related to the matrix metalloproteinase (MMP) pathway.[32] They found that, following tissue injury, treatment with rofecoxib up-regulated the MMP1 (matrix metalloproteinase 1), MMP3 (matrix metalloproteinase 3), PLAT (tissue plasminogen activator), and IL8 (interleukin 8) genes and down-regulated the CD36 (CD36 antigen), VIP (vasoactive intestinal peptide), VIPR1 (vasoactive intestinal peptide receptor I), and TIMP3 (tissue inhibitor of metalloproteinase 3) genes.[32] Treatment with ibuprofen also upregulated MMP1 and MMP3, compared with placebo treatment where gene expression remain unchanged.[32] The significance of the MMP1 and MMP3 genes is that they are associated with inflammation and are likely to have roles in atherogenesis.[32] Therefore, we hypothesized that these genes may have polymorphisms predictive of coxib related cardiovascular toxicity.

Although no direct relationship between NSAIDs or COX-2 inhibitors and MMP9 have been reported, MMP9 is believed to have a role in coronary artery disease. Zhang et al. reported that a particular polymorphism (-1562C/T) in the promoter region of the gene was associated with the severity of coronary atherosclerosis.[38] Therefore, we also included the MMP9 gene in our investigation.

1.5.4 The Metabolism Genes of NSAIDs and COX-2 inhibitors

A variety of hepatic P450 enzymes including CYP2C9, CYP2C8 and CYP3A4 are involved in the metabolism of NSAIDs and COX-2 inhibitors.[39] Our study focuses on the two main COX-2 selective inhibitors, rofecoxib and celecoxib, which are metabolized differently. The metabolism of rofecoxib is complex and is primarily performed by the cytosolic enzymes of the liver with a minor role by CYP2C9.[39] However, CYP2C9 is responsible for approximately 70-90% of the metabolism of celecoxib.[39] Furthermore, it has been demonstrated that the hydroxylation of celecoxib was decreased by approximately 50% in livers that were heterozygous for **CYP2C9*1/*3** versus homozygous for CYP2C9*1/*1.[40] In addition to celecoxib, CYP2C9 plays a significant role in the clearance of other NSAIDs including meloxicam and ibuprofen.[39] However, not all traditional NSAIDS are cleared by CYP2C9. Like rofecoxib, naproxen and diclofenac are metabolized by non-P450 mechanisms.[39] Although the mechanisms of drug metabolism vary significantly among NSAIDs and COX-2 inhibitors, evidence of variation in drug clearance according to CYP2C9 **genotype** for celecoxib prompted the inclusion of the CYP2C9 gene in our study.

1.6 Additional SNPs of Interest

In addition to these seven candidate genes, we investigated 21 additional SNPs in 12 genes for their potential to interact with NSAIDs to produce adverse cardiovascular events. These candidate SNPs are in genes that have been associated with conditions or processes involved in the development of coronary heart disease or thrombotic events including hypertension, hyperlipidemia, diabetes, obesity, atherosclerosis and aging (Table 1.1). The relative risks of these polymorphisms are generally modest. However, we chose to investigate these SNPs based on our hypothesis that their interaction with NSAIDs or COX-2 inhibitors may further increase cardiovascular risk.

In recent years, the role of C-reactive protein (CRP) in cardiovascular disease and metabolic syndrome has been a popular focus of investigation.[41] The

protein is a known inflammatory **marker** and associations have been found with atherosclerosis, ischemic stroke, metabolic syndrome and coronary artery disease.[41-44] Furthermore**,** several studies have shown that genetic variation within the CRP gene is associated with circulating CRP concentration.[41-44] In particular, rs1205 located in **exon** 2 of the CRP gene has been linked to decreased levels of serum CRP in several populations.[42, 45] However, some studies suggest that neither baseline CRP levels nor variation within the CRP gene are associated with cardiovascular risk factors including hypertension, blood pressure and metabolic syndrome.[41, 46, 47] Despite this, we felt variation in CRP may modulate cardiovascular risk in NSAID exposed patients and rs1205 was investigated in our study.

The angiotensinogen (AGT) gene has a well characterized role in controlling blood pressure via the renin-angiotensin system.[48] Among polymorphisms within this gene, previous research has provided evidence of association between SNP rs943580 and echocardiographic **phenotypes**, and rs699 (M235T) and myocardial infarction, coronary heart disease and stroke.[48-52] These studies were mostly small and many were conducted in specific populations including users of angiotensin converting enzyme (ACE) inhibitors, hypertensive patients, and a small subset of the Spanish population with a high prevalence of cardiovascular disease.[48-52] Additional research suggests that an interaction between the AGT M235T polymorphsim and other genes of the renin-angiotensin system, including the APOE, AGTR1 and ACE (I/D) genes may promote coronary artery disease. [53, 54] Therefore, we included rs943580 and rs699 of the AGT gene in our study.

The interleukin-18 gene (IL18) is implicated in atherosclerosis and baseline levels of circulating interleukin-18 have been shown to be predictive of cardiovascular mortality.[55] We selected two tagging SNPs of interest, rs543810 and rs360722, for our study**.**

Genetic variation in the klotho (KL) gene has been associated with premature aging in humans, atherosclerosis and the risk of early-onset occult

coronary artery disease. [56, 57] A tagging SNP (rs211247) near the KL gene was selected for our study.

Previous studies of the estrogen receptor 1 (ESR1 or ESRα) and estrogen receptor 2 (ESR2 or ESRβ) genes have shown that genetic variation in these genes may be linked with longitudinal blood pressure, coronary artery disease and myocardial infarction.[58-60] We selected four SNPS, rs3853248 and rs11155814 in ESR1 and rs7154455 and rs3020450 for our study**.**

A recent study investigating 103 candidate genes for coronary artery disease in a founder population in the Saguenay Lac St-Jean region of Quebec identified rs5370 in the endothelin-1 (EDN1) gene as a predictor of high density lipoprotein (HDL) cholesterol concentration in women.[61] We included this polymorphism as well as two additional SNPS, rs9369217 and rs9380973 in our study.

The Pare et al. study provided evidence of an association between the apolipoprotein E (APOE) gene and low density lipoprotein (LDL) cholesterol.[61] There are three major isoforms of APOE that are coded by three alleles of the APOE gene called, ε2, ε3, and ε4.[62] These alleles can be distinguished by genotyping two SNPS, rs429358 and rs7412, which are included in our study.

The paraoxonase (PON1) gene is implicated in myocardial infarction and stroke.[63, 64] We selected one tagging SNP (rs854542) downstream of this gene for inclusion in our study.

Recently, it has been shown that the resistin (RETN) gene plays a role in inflammation and inflammation related diseases.[65] This role was discovered during several studies exploring the role of this protein in obesity and type-2 diabetes.[65, 66] Therefore, we included a SNP (rs3219177) in **intron** 2 of the RETN gene**.**

Genetic variation in the phopholipase A2, group VII (PLA2G7) gene has been associated with hypertension and future cardiovascular events in patient with coronary artery disease.[67, 68] We included SNP rs1805018 of the PLA2G7 gene which was studied by Nino et al. who found that individuals homozygous for the minor allele were significantly less likely to be hypertensive.[68]

Finally, two recent studies have found that a region in **chromosome** 9 known as 9p21 is associated with coronary heart disease.[69, 70] McPherson et al. found that the common alleles of SNPs rs10757274 and rs2383206 were associated with the risk of coronary artery disease while Helgadottir et al found that several other correlated SNPs, rs133040, rs2383207, rs10116277, and rs10757278 within the same region were associated with myocardial infarction.[69, 70] In the McPherson et al. study the reported hazard ratios for coronary heart disease were small ranging from 1.18 to 1.29 for carriers of the major allele (G), however, we wondered whether exposure to NSAIDs may further increase cardiovascular risk.[70] We investigated two alleles from this region, rs10757274 and rs2383206.

To date there is some evidence that genetic variation has implications for the therapeutic efficacy of NSAIDs and COX-2 inhibitors. Furthermore, several genetic variants have been associated with cardiovascular health. Therefore, it is not unreasonable to hypothesize that genetic variation may affect the cardiovascular safety of NSAIDs.

1.7 Rationale and Objectives

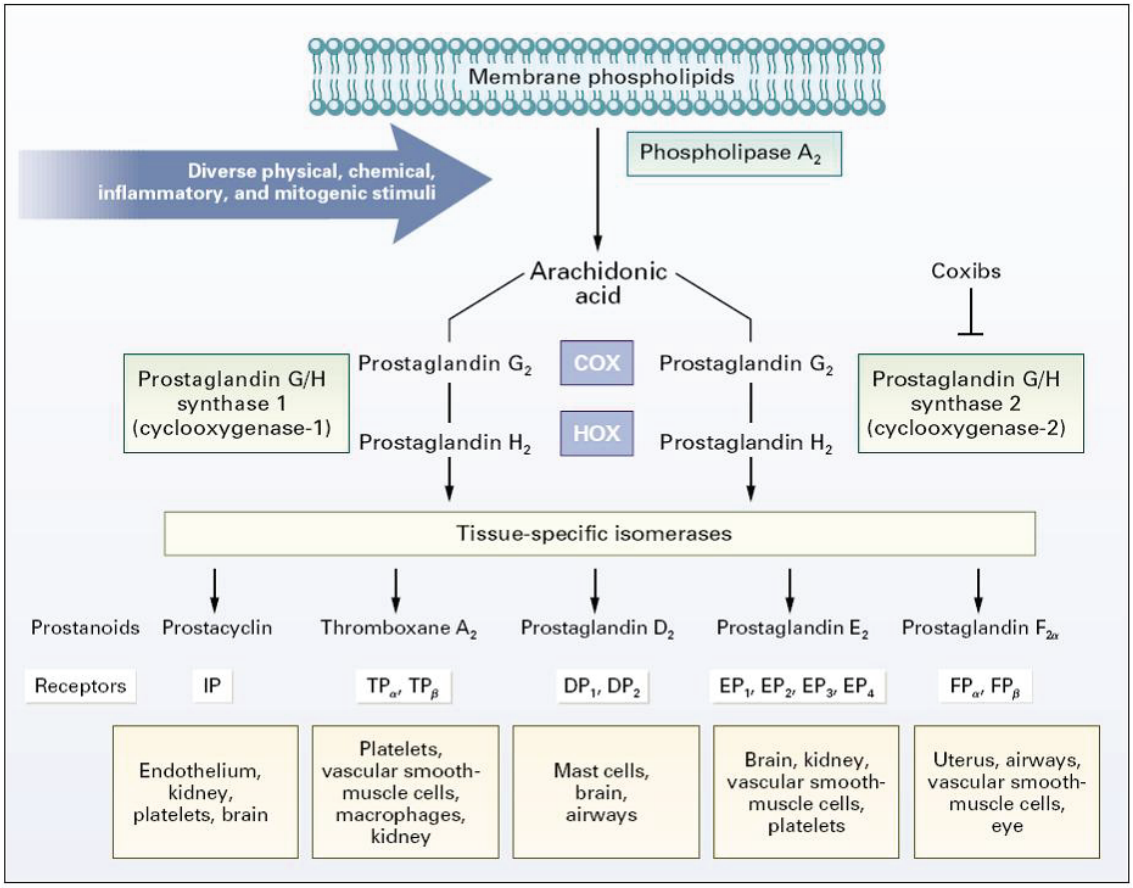
The cardiovascular safety of selective COX-2 inhibitors and NSAIDs is of continuing debate and clinical investigations are ongoing. To date, there are no published studies examining the gene-drug interaction between NSAIDs and genetic polymorphisms in patients who have had a myocardial infarction.

We have the opportunity to investigate a group of patients who were admitted with a myocardial infarction (MI) or unstable angina (UA) to the Recurrence et inflammation dans les syndromes coronariens aigus (RISCA) study between 2000 and 2002.[71] The primary purpose of this study was to investigate predictors of the recurrence of coronary events with particular focus on inflammatory markers. However, most patients provided a genetic sample and complete medication data upon entering this study and consented to its use in additional investigations. Therefore, these patients also provide an excellent opportunity to investigate our study question. Furthermore, due to the worldwide

withdrawal of rofecoxib, the opportunity to investigate genetic samples in rofecoxib users suffering a myocardial infarction is likely to be unique to the RISCA cohort. However, genetic samples are being collected in the PRECISION study, a large prospective randomized controlled trial investigating celecoxib, ibuprofen and naproxen, which may also provide some insight into gene-NSAID interactions.[72] The PRECISION study is powered for cardiovascular outcomes and will be completed in 2011.

The objective of this study is to utilize the RISCA cohort of individuals admitted to hospital for MI or UA to investigate candidate genes for single nucleotide polymorphisms (SNPs) that may be predictive of adverse cardiovascular events upon use of a NSAID or COX-2 inhibitor. By identifying genetic markers that may predict increased susceptibility to NSAID or COX-2 inhibitor associated cardiovascular events, it may be possible to prospectively identify those patients who according to their genotype are at risk of a cardiovascular event and those whose risk remains unchanged.

Figure 1.1 The role of cyclooxygenase-1 (COX-1) and cycloxygenase-1 (COX-2) in the production of prostaglandins[73]



FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med. Aug 9 2001;345(6):433-442. Copyright © 2007 Massachusetts Medical Society. All rights reserved.

Arachidonic acid is metabolized by the cyclooxygenase 1 and cyclooxygenase 2 enzymes produced by the constitutively expressed prostaglandin endoperoxide synthase 1 (PTGS1) gene and the inducible endoperoxide synthase 2 (PTGS2) gene, respectively. The enzymes facilitate oxygenation of arachidonic acid to produce prostaglandin G_2 which is subsequently reduced to prostaglandin H_2. Prostaglandin H_2 is then metabolized into several prostanoids including prothrombotic thromboxane A_2 and the anti-thrombotic prostacyclin also known as prostaglandin I_2.

Table 1.1: Candidate SNPs selected by literature review.

Candidate SNP	Gene or Region	Gene Symbol	References
rs943580	Angiotensinogen	AGT	48-54, 74, 75
rs699	Angiotensinogen	AGT	48-54, 74, 75
rs429358*	Apolipoprotein E	ApoE	62, 76
rs7412*	Apolipoprotein E	ApoE	62, 76
rs10757274	Chromosome 9	Chr9p21.3	69, 70
rs2383206	Chromosome 9	Chr9p21.3	69, 70
rs1205	C-Reactive Protein	CRP	41-44, 46, 47, 77-79
rs9369217	Endothelin-1	EDN1	61, 80
rs9380973	Endothelin-1	EDN1	61, 80
rs5370	Endothelin-1	EDN1	61, 80
rs3853248	Estrogen Receptor 1	ESR1	58-60
rs11155814	Estrogen Receptor 1	ESR1	58-60
rs7154455	Estrogen Receptor 2	ESR2	58-60
rs3020450	Estrogen Receptor 2	ESR2	58-60
rs543810	Interleukin-18	IL18	55
rs360722	Interleukin-18	IL18	55
rs211247	Klotho	KL	56, 57, 81-83
rs854542	Paraoxonase-1	PON1	63, 64, 84
rs1805018	Phospholipase A2, group VII	PLA2G7	67, 68
rs3219177	Resistin	RETN	65, 66
rs13306848*	Thrombomodulin	THBD	85-90

*These SNPs were not successfully genotyped due to genotyping failure or error.

1.8 References

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Chapter 2

Study Design and Methods

2.1 Study population

From 2000 to early 2002, the RISCA (Récurrence et inflammation dans les syndromes coronariens aigus) study recruited 1210 participants admitted to hospital for unstable angina (UA) or myocardial infarction (MI) from four tertiary and four community hospitals, seven of which are located in Quebec (Hopital Laval, Thetford Mines, Sagamie, Grand-Portage, Notre Dame, Drummondville, Montreal General) and one in New Brunswick. Participating centers were intentionally chosen to represent a balanced mix of university and community based centers. Attempts were made to recruit subjects consecutively in each center and a register of patients admitted but not recruited was maintained.

Subjects were recruited within 24 hours of symptom onset, according to a specified clinical definition for UA and MI. MI was defined as characteristic discomfort or pain with an elevation of creatine kinase-MB (CK-MB) to ≥ 1.5 times the upper normal limit and included both incident and recurrent cases. UA was defined as either one episode lasting ≥10 min, or ≥2 episodes lasting ≥5 min within 24h, or characteristic discomfort or pain at rest with minimal exertion, and at least one of these features: ECG changes; cardiac troponin I or T values in the positive range for MI; history of MI or coronary revascularization; previous coronary angiogram with at least 50% stenosis; previous non-invasive test showing myocardial ischemia or evidence of MI; known peripheral vascular disease; known non hemorrhagic cerebrovascular disease; or the presence of diabetes. Principal investigators of the RISCA study confirmed diagnoses and in the case of ambiguities an outside clinical researcher was used for adjudication. Of the 1210 recruits, 747 were admitted for MI and 416 for UA, with the remainder not meeting either clinical definition.

Upon admission to the study, a comprehensive Case Report Form was used to collect basic demographic and medical data, risk factors, and diagnostic information, including recurrent ischemia, cardiac function, and use of invasive cardiac procedures. All baseline medications were recorded. NSAID use and the name of the drug being used were recorded if treatment was taken within the last 10 days. NSAIDs prescribed *pro re nata* (*prn*) were not recorded. Exposure to

rofecoxib or celecoxib or other NSAIDs was determined from baseline medication data upon admission into the RISCA study. These forms have been systematically verified and on-site visits were made to all centers to clear up ambiguities and triple verify the data by detailed chart review. All final diagnoses were confirmed.

For the genetic component of the RISCA study, blood samples were collected from 1084 consenting subjects within 24 hours of symptom onset. A computerized blood storage system was used, ensuring precise and instantaneous inventory information and efficient retrieval while maintaining patient anonymity. The genetic samples required for this study were shipped from the University of Laval to McGill University where they were stored at -80C.

Given that the RISCA cohort has excellent phenotyping as well as genotyping potential, we examined its suitability to investigate candidate genes predictive of myocardial infarction and unstable angina upon NSAID exposure.

2.2 The case-only study design

As sequencing of the human genome becomes more accessible and affordable, the methods of human genome epidemiology continue to evolve. In 1994, Piegorsch et al. proposed the **case-only study design** to investigate gene-environment interactions.[1] In this design, all individuals included in the sample are affected by the disease (cases) and each individual is assessed for the presence or absence of a genotype (G) and an exposure (E).[2] The odds ratio (OR) derived from this study can be interpreted as "the odds of E given the presence of G divided by the odds of E given the absence of G" or "the odds of G given the presence of E divided by the odds of G given the absence of E." This OR indicates the multiplicative interaction between genotype and exposure in causing disease (Table 2.1).[2] For example, an odds ratio of 3 suggests that the relative risk of MI or UA with the susceptible genotype and exposure to an NSAID is 3 times greater than the relative risk of MI from genotype alone multiplied by the relative risk of MI from exposure alone.[2] However, the validity of this estimate relies on the assumption that the genotype and exposure are independent of one another. If

this assumption holds true, the case-only design is more efficient and gives more precise estimates than the case-control design.[3] This gain in efficiency is a direct result of removing the variance introduced by controls.[2] Furthermore, it eliminates the difficult task of selecting or recruiting a control group and avoids the potential biases that may be introduced by selecting inappropriate controls.[2]

There are limitations to the case-only design. First and foremost, violation of the independence assumption compromises the validity of the study design. Secondly, sample size and power estimates are a complex function of multiple factors, many of which are difficult to estimate.[3, 4] Another limitation of studies using single nucleotide polymorphisms (SNPs) is the issue of multiple testing. For each gene there can be hundreds of SNPs and while high throughput testing can allow for all of them to be tested, the large number of results makes it difficult to discern true from false positives. Several solutions have been proposed in past literature; these include retaining a study sample to retest positive results, Bayesian analysis to incorporate prior information, and the Bonferroni correction.[4] Aside from the Bonferroni correction which is very conservative, these approaches are not feasible for this study. Given the hypothesis generating nature of this study, p-values were not adjusted. However, multiple testing was considered when discussing positive results. Furthermore, at a p-value level of 0.05 we would expect 5% or at least 5 or 6 SNPs of 115 SNPs tested to be significant by chance; therefore, significant SNPs in excess may be considered indicative of non-spurious findings.

2.3 Candidate gene selection

Genes were individually assessed for the plausibility that they may contain single nucleotide polymorphisms (SNPs) that, when in the presence of NSAIDs, are associated with the occurrence of MI. Because this investigation is exploratory and financial resources were limited, we used a candidate gene approach as opposed to a **genome**-wide analysis. The candidate gene approach uses previous literature and knowledge to identify genes or polymorphisms that are most likely to be associated with the outcome of interest.[5] The criteria used include biological

plausibility, positional data, prior association studies, and prior animal studies.[5] Although the candidate gene approach is considered by many to be an "imprecise art" and genome-wide analysis is gaining popularity, the candidate gene approach does avoid the cost and interpretation problems of genome-wide scans which test hundreds of thousands of polymorphisms.[5]

We identified candidate genes with a focused literature review of peer reviewed abstracts and publications. Genes were considered candidates for investigation if there was published evidence that they are regulated by COX-2 inhibitors, associated with the cyclooxygenase pathway or associated with cardiovascular events.

The first two candidates were selected based on the two main protein targets of non-steroidal anti-inflammatory drugs, the COX-1 and COX-2 enzymes. As discussed earlier, these proteins are encoded by the prostaglandin-endoperoxide synthase 1 (PTGS1) and the prostaglandin-endoperoxide synthase 2 (PTGS2) gene, respectively. Additional genes were selected based on the biological plausibility of interaction with NSAIDs or COX-2 inhibitors to increase the risk of cardiovascular events. PTGIS was included because of evidence of coupling with the COX-2 enzyme, its role in platelet aggregation, vasodilation, vascular remodeling, angiogenesis and apoptosis as well as evidence that NSAIDs and COX-2 inhibitors may reduce the production of PGI_2.[6] MMP1, MMP3 and MMP9 were selected for their association with the processes of coronary artery disease including inflammation and atherogenesis and evidence of regulation by COX-2 inhibitors.[7, 8] Finally, CYP2C9 was included for its role in the metabolism of several NSAIDs, particularly celecoxib.[9] For each gene selected, we selected a group of single nucleotide polymorphisms to provide complete coverage of the gene. In addition to these seven candidate genes, we included 21 additional SNPs of interest based on previous studies suggesting they have a role in the conditions or processes involved in the development of coronary artery disease. (See Chapter 1, Table 1.1)

2.4 SNP selection

SNP genotype data was extracted from the International HapMap Project publicly available at www.hapmap.org. This website is a comprehensive database of genetic and SNP genotype data collected from "four populations with African, Asian and European ancestry."[10] For each candidate gene, the location of each gene was identified by searching the HapMap database for the gene symbol (PTGIS, PTGS1, PTGS2, MMP1, MMP3, MMP9 and CYP2C9). Based on the search results, SNP genotype data was downloaded starting 5000 base pairs (bp) before the start of the gene and ending 5000bp after the end of the gene. Inclusion of the flanking regions provides complete SNP coverage of each gene and provides SNP data for any potential regulatory regions that may lie outside of the transcribed gene region.

Downloaded SNP genotype data were imported into Haploview, publicly available at http://www.broad.mit.edu/mpg/haploview/.[11] The Haploview program provides users with an interface to perform **haplotype** analysis as well as SNP tagging. The Tagger function in Haploview was used to select a subset of SNPs representative of the entire gene. This is done by grouping markers (SNPs) into "bins" that are highly correlated and selecting one SNP to be tested from each "bin". We selected pairwise tagging and a minimum correlation coefficient (r^2) of 0.8 to ensure that within each bin every SNP was correlated with every other SNP at an r^2 level of 0.8. Furthermore, all SNPs included in the tagging process had a minimum minor allele frequency of 0.05.

2.5 Patient selection

We designed a case-only study, nested within the RISCA study. Among 1210 patients, 671 subjects admitted for MI and 376 patients admitted for UA consented to a genetic sample. Of these, 70 individuals admitted for MI reported treatment with a NSAID (n=26 rofecoxib, n=33 celebrex, n=11 other NSAID) and 45 admitted for UA reported treatment with a NSAID (n=17 rofecoxib, n=16 celebrex, n=7 other NSAID) (Figure 2.1). All subjects admitted for MI or UA who reported treatment with an NSAID were included as the "exposed" group.

Among 932 subjects who did not report treatment with an NSAID, 345 were selected as the "unexposed" comparison group. This comparison group included three subjects matched on age (+/- 10 years), sex and hospital center for every "exposed" subject.

Optimal matching was conducted using a publicly available match macro for SAS (http://cancercenter.mayo.edu/mayo/research/biostat/sasmacros.cfm). For one patient from the New Brunswick center, only one unexposed patient was available using the 10 year age strata. In order to preserve this case, we manually matched her to two additional unexposed female patients from the same center.

2.6 Genotyping

Genetic samples from selected patients were delivered to the McGill University and Genome Quebec Innovation Centre. Genotyping of all selected SNPs was performed at Genome Quebec using the Sequenom iPLEX Gold Assay (Sequenom, Cambridge, MA).

2.7 Analysis

Exploratory descriptive analysis compared the frequencies of selected single nucleotide polymorphisms (SNPs), among individuals exposed to rofecoxib or celecoxib or other NSAIDs, with those who did not report treatment with a NSAID or COX-2 inhibitor at baseline. All statistical analyses were done in SAS.

A case-only analysis assuming independence of genotype and NSAID exposure was used to assess potential gene-drug interactions. For this analysis, conditional logistic regression was used for comparisons of genotype frequencies among individuals exposed to NSAIDs upon admission for their acute coronary syndrome versus a comparison group matched on age (+/-10 years), sex and hospital center who did not report treatment with a NSAID at baseline. The homozygous major allele was used as a reference category for the estimates of case-only odds ratios and 95% confidence intervals.

In a planned subgroup analysis, we limited our case-only study group to individuals exposed to COX-2 inhibitors and their matched unexposed comparison group. P-values were not corrected for multiple testing.

Table 2.1: Calculating the case-only odds ratio (OR)

a. Case-Only Study (D+):

	G+	G–
E+	a_1	a_2
E–	c_1	c_2

b. Total Cohort (D+ and D–):

	G+	G–
E+	N_{11}	N_{01}
E–	N_{10}	N_{00}

$$\text{Case-Only OR} = \frac{a_1 c_2}{a_2 c_1} \quad \text{Term I}$$

$$\text{G-E OR} = \frac{N_{11} N_{00}}{N_{01} N_{10}} \quad \text{Term II}$$

c.

$$G_E \times E_{RR} = \frac{RR_{GE}}{RR_G * RR_E} = \frac{\frac{a_1 / N_{11}}{c_2 / N_{00}}}{\frac{c_1 / N_{10}}{c_2 / N_{00}} * \frac{a_2 / N_{01}}{c_2 / N_{00}}} = \frac{\left(\frac{a_1 c_2}{c_1 a_2}\right)}{\left(\frac{N_{11} N_{00}}{N_{10} N_{10}}\right)}$$

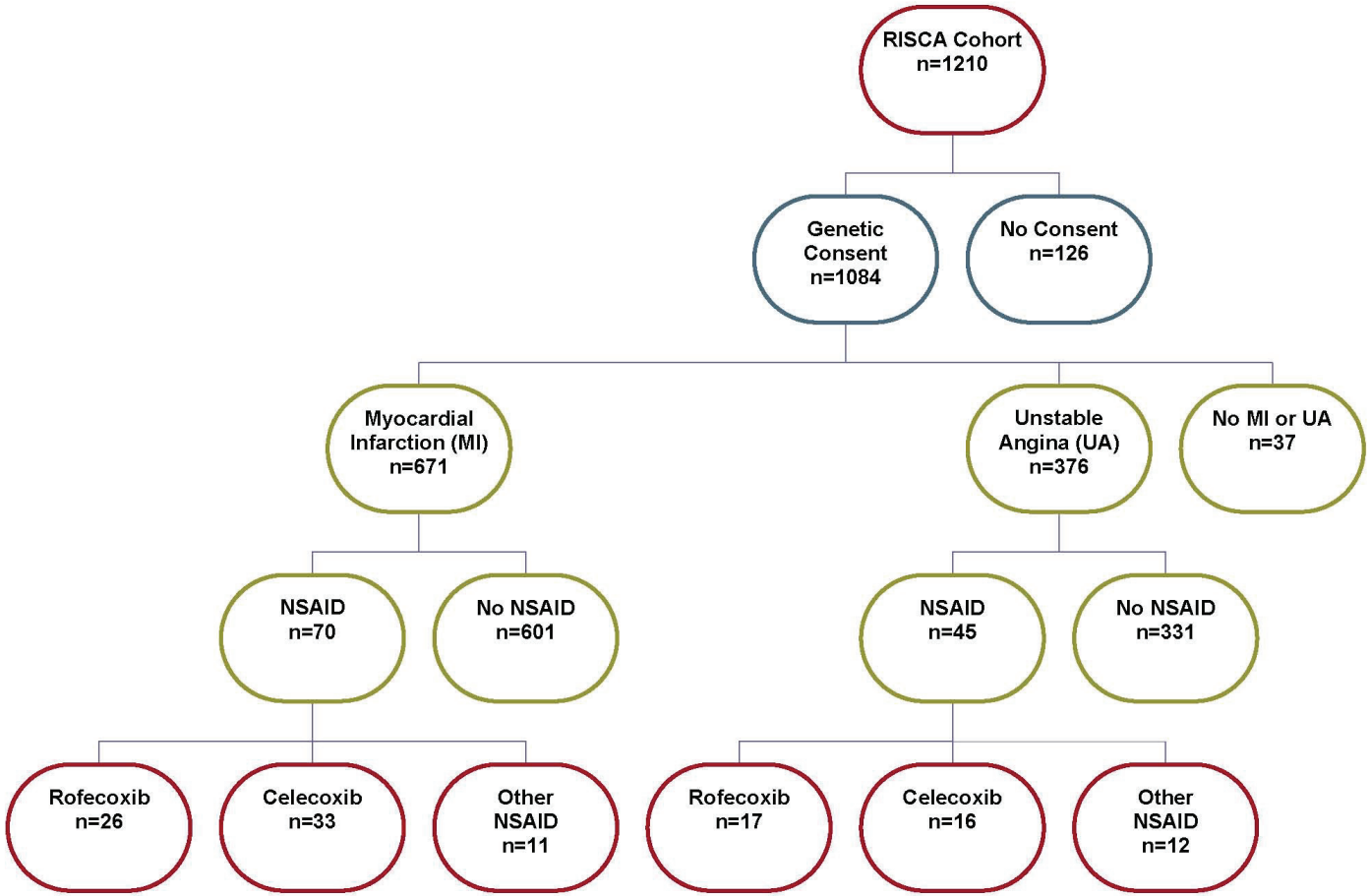
Term I

Term II

Gatto N, Campbell U, et al. Further development of the case-only design for assessing gene-environment interaction: evaluation of and adjustment for bias. Int J Epidemiology. 2004:33:1014-1024, by permission of Oxford University Press (Licence Number 1861500266877).

The case-only odds ratio is an estimate of the multiplicative interaction of genotype and environment in causing disease. When genotype and exposure are independent in the total cohort (Term II = 1), the case-only odds ratios is equivalent to the gene-environment interaction risk ratio (G X E_{RR}).

Figure 2.1: Selection of exposed cases from the RISCA cohort



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Chapter 3

Manuscript

3.1 Preface

This chapter contains a manuscript that will be submitted for publication.

The contribution of authors is listed separately on page vi. As a supplement to the manuscript, additional results and discussion are included in Chapter 4 of this thesis.

3.2 Manuscript

Title:

Genetic Polymorphisms and the Cardiovascular Risk of COX-2 Inhibitors

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3.2.1 Abstract

Title: Genetic Polymorphisms and the Cardiovascular Risk of COX-2 Inhibitors

Background:
The cardiovascular safety of cyclooxygenase-2 (COX-2) selective non-steroidal anti-inflammatory drugs (NSAIDs) is of concern, although the majority of users remain free of adverse outcomes. A gene-drug interaction may contribute to the variation in individual response to COX-2 inhibitors.

Methods:
In a case-only study of 460 patients selected from a cohort admitted for an acute coronary syndrome including myocardial infarction and unstable angina, we genotyped 115 single nucleotide polymorphisms (SNPs). We identified 115 exposed patients who reported treatment with rofecoxib (n=43), celecoxib (n=49), or another NSAID (n=23) within 10 days prior to hospital admission and selected 345 unexposed patients matched for age, sex and hospital center.

Results:
We observed statistically significant gene-drug interactions between NSAID exposure and 14 SNPs. Furthermore, when the study group was limited to subjects exposed to rofecoxib (n=43) or celecoxib (n=49) and the matched unexposed subjects (n=276), the association between coxib exposure and genotype strengthened for 4 SNPs. Using the homozygous major allele as a reference, the homozygous minor allele yielded statistically significant case-only odds ratios for a SNP in the C-Reactive Protein (CRP) gene (OR=3.6; 95% confidence interval [CI], 1.6 - 7.9; P=0.001) as well as two SNPs in the cyclooxygenase-1 (COX-1) gene (OR=6.9; 95% CI, 1.4 - 35.7; P=0.02 and COR=6.9; 95% CI, 1.3 - 35.6; P=0.02). Within the Klotho gene, the heterozygote of one SNP yielded a statistically significant case-only odds ratio of 2.3 (95% CI, 1.4-4.0; P=0.002).

Conclusions:

Genetic polymorphisms within the COX-1, CRP and Klotho genes are candidates for gene-drug interactions influencing the cardiovascular outcomes of users of NSAIDs and COX-2 inhibitors. These findings suggest that genetic susceptibility may contribute to coronary instability in some users of this class of drugs.

3.2.2 Background

Selective cyclooxygenase-2 (COX-2) inhibitors are effective for the treatment of arthropathic, menstrual, and acute pain but are associated with an increase in adverse cardiovascular outcomes. The potent selective COX-2 inhibitor, rofecoxib (Vioxx) was voluntarily withdrawn from the worldwide market because several studies demonstrated an increased cardiovascular risk.[1, 2] Furthermore, other COX-2 inhibitors have also been associated with an increase in adverse cardiovascular outcomes.[3, 4] These studies have raised concern of a class effect, prompting further investigation into the safety of not only selective COX-2 inhibitors but all non-steroidal anti-inflammatory drugs (NSAIDs).

Although an increase in cardiovascular risk has been observed among users of COX-2 inhibitors, the absolute rate of patients experiencing a thrombotic event in the APPROVe trial was low (1.5 per 100 patient years) suggesting that the majority of patients were treated without incident.[2] The biological mechanism that triggers these cardiovascular events is still unclear. The most common hypothesis centers on a potential disequilibrium in prostaglandin synthesis between prothrombotic thromboxane A2 (TXA_2) and antithrombotic prostacyclin (PGI_2), which are regulated by the COX-1 and COX-2 enzymes respectively.[5] However, low-dose aspirin does not appear to attenuate the risk suggesting it is not solely due to a prothrombotic state.[5] Alternatively, some NSAID users may be genetically susceptible to a cardiovascular event while other users have a cardiovascular risk that remains unchanged.

Recent studies have shown that genetic variants in key prostaglandin metabolic genes may lead to different levels of therapeutic response to NSAIDs as well as variation in prostaglandin synthesis and the development of hypertension.[6-10] Therefore, we investigated whether variation in select candidate genes might explain some of the variability in adverse cardiac outcomes. While our study focuses on the two enzyme targets of NSAIDs, COX-1 and COX-2; we also examined several other candidate genes including MMP1, MMP3 and MMP9 of the matrix metalloproteinase pathway and CYP2C9 (a cytochrome P450 enzyme) as well as candidate SNPs previously associated with inflammation,

thrombosis or cardiovascular disease processes. Thus, this study investigates potential gene-drug interactions between candidate single nucleotide polymorphisms (SNPs) and the use of NSAIDs or COX-2 inhibitors that may contribute to cardiovascular risk.

3.2.3 Methods

Cases

We studied 460 (38%) patients from the RISCA (Recurrence et inflammation dans les syndromes coronariens aigus) cohort admitted for an acute coronary syndrome (ACS) including myocardial infarction (MI) and unstable angina (UA)[11]. Patients were drawn from four tertiary and four community hospitals, seven of which are located in Quebec and one in New Brunswick. Patients were recruited within 24 hours of symptom onset, according to a specified clinical definition for UA and MI. MI was defined as characteristic discomfort or pain with an elevation of creatine kinase-MB (CK-MB) to ≥ 1.5 times the upper normal limit or troponin considered positive for MI. This included both incident and recurrent cases. UA was defined as either one episode lasting ≥10 min, or ≥2 episodes lasting ≥5 min within 24h, or characteristic discomfort or pain at rest or with minimal exertion, and at least one of these features: ECG changes; cardiac troponin I or T value considered positive for myocardial necrosis with CK/CKMB under the threshold definition of MI; history of MI or coronary revascularization; previous coronary angiogram with at least one artery with ≥50% stenosis; previous non-invasive test showing myocardial ischemia or evidence of MI; known peripheral vascular disease; known non hemorrhagic cerebrovascular disease; or the presence of diabetes. Principal investigators of the RISCA study confirmed diagnoses and in the case of ambiguities an outside clinical researcher was used for adjudication

Upon admission to the cohort, a comprehensive Case Report Form was used to collect basic demographic and medical data, risk factors, and diagnostic information including recurrent ischemia, cardiac function, and use of invasive cardiac procedures. All baseline medications were recorded. NSAID use and the

specific drug being used were recorded if treatment was taken within the last 10 days. NSAIDs prescribed *prn* were not recorded. Aspirin treatment ≤325mg was recorded separately. These forms have been systematically verified and on-site visits were made to all centers to clear up ambiguities and triple verify the data by detailed chart review. Informed consent for the collection of blood samples for genetic testing was obtained from all participants.

Candidate Gene Selection

We identified candidate genes from a literature review of peer reviewed abstracts and publications. Genes were considered candidates for investigation if there was published evidence that they are regulated by COX-2 inhibitors, associated with the cyclooxygenase pathway or associated with cardiovascular events or risk factors.

The first two candidates were the COX-1 and COX-2 enzymes. COX-1 is encoded by the constitutively expressed prostaglandin-endoperoxide synthase 1 (PTGS1) gene and produces prostaglandins involved in the regulation of stomach mucosa, platelet aggregation, and kidney function.[12] COX-2 is encoded by the inducible PTGS2 gene, is rapidly induced by inflammatory cytokines and mitogens, and is believed to produce the majority of prostaglandins involved in inflammation and cancer.[12] These enzymes are mediators of key prostaglandins involved in vascular homeostasis including prostacyclin and thrombaxane A2.

Recent research supports the hypothesis that variants in the PTGS1 and PTGS2 genes may influence the efficacy or safety of NSAIDs or COX-2 inhibitors. For example, one study showed that the level of PTGS2 expression and the degree of pain relief experienced upon treatment with rofecoxib and ibuprofen varied by allele group.[7] Furthermore, there is evidence that several genetic variants in the PTGS1 gene alter the metabolism of arachidonic acid to Prostaglandin H2 (PGH_2); the precursor to prostacyclin, thromboxane and several other prostaglandins.[9] Moreover, inhibition of PGH_2 by aspirin varied by allele group at two SNPs located within the PTGS1 gene.[8]

Further down the prostaglandin metabolic pathway, the Prostaglandin I_2 Synthase gene (PTGIS) facilitates the isomerization of PGH_2 to prostacyclin. Since prostacyclin is the most potent inhibitor of platelet aggregation and a strong vasodilator, genetic variation within the PTGIS gene may affect cardiovascular health. Indeed, genetic variants in PTGIS have shown association with hypertension.[10]

Additional genes were selected based on the biological plausibility of interaction with NSAIDs or COX-2 inhibitors to increase the risk of cardiovascular events. These include genes within the matrix metalloproteinase pathway (MMP1, MMP3 and MMP9) and genes involved in the metabolism of COX-2 inhibitors (CYP2C9).[13-15] These selections are supported by evidence of rofecoxib and ibuprofen altering the gene expression of MMP1 and MMP3, a putative role of MMP9 in coronary heart disease, and the role of CYP2C9 in the metabolism of celecoxib.[13, 15, 16] In total, we identified 7 prospective candidate genes (PTGS1, PTGS2, PTGIS, MMP1, MMP3, MMP9 and CYP2C9) and selected 84 tagging SNPs to investigate.

Finally, we selected 21 additional SNPs in 12 genes for their potential to interact with NSAIDs to cause adverse cardiovascular events. These candidate SNPs are in genes that have been associated with conditions or processes involved in the development of coronary heart disease or thrombotic events including hypertension, hyperlipidemia, diabetes, obesity, atherosclerosis and aging. These include the following genes: C-Reactive Protein (CRP), Angiotensinogen (AGT), Interleukin-18 (IL18), Klotho (KL), Estrogen Receptor 1 (ESR1), Estrogen Receptor 2 (ESR2), Endothelin 1 (EDN1), Apolipoprotein (APOE), paraoxonase 1 (PON1), Resistin (RETN), Phospholipase A2, group VII (PLA2G7), thrombomodulin (THBD) and a recently identified region of chromosome 9 known as 9p21.3. (Table 3.1)

SNP Selection and Genotyping

SNP genotype data was extracted from the International HapMap Project publicly available at www.hapmap.org. For each candidate gene (PTGIS, PTGS1,

PTGS2, MMP1, MMP3, MMP9 and CYP2C9), SNP genotype data was included starting 5000 base pairs (bp) before the start of the gene and ending 5000 bp after the end of the gene. Inclusion of the flanking regions provides more complete SNP coverage of each gene and provides SNP data for any potential regulatory regions that may lie outside of the transcribed gene region.

The Tagger function in Haploview (http://www.broad.mit.edu/mpg/haploview) was used to select a subset of SNPs that would best capture each gene. We performed tagging with a minimum correlation coefficient (r^2) of 0.8 to ensure that within each bin every SNP was correlated with every other SNP at an r^2 level of 0.8. All SNPs included in the tagging process had a minimum minor allele frequency (MAF) of 0.05.

Additional candidate SNPs were identified through literature review (Table 3.1). Genotyping was performed at the McGill University and Genome Quebec Innovation Centre using the Sequenom iPLEX Gold Assay (Sequenom, Cambridge, MA).

Statistical Analysis

We used the case-only study design to investigate gene-environment interactions.[17] In this design, all individuals included in the sample are cases and each individual is assessed for the presence or absence of a genotype and an exposure.[17, 18] The resulting odds ratio derived from this study is indicative of the multiplicative interaction between genotype and exposure. For example, an odds ratio of 3 suggests that the relative risk of MI or UA with susceptible genotype and exposure to an NSAID is 3 times greater than the relative risk of MI from genotype alone multiplied by the relative risk of MI from exposure alone.[18] This design relies on the assumption of independence between genotype and exposure and provides greater statistical efficiency by eliminating the variance and potential biases associated with controls.[18]

We used conditional logistic regression to compare genotype frequencies among individuals exposed to NSAIDs upon admission for their acute coronary syndrome versus a comparison group matched on age, sex and hospital center

who did not report treatment with a NSAID at baseline. The homozygous major allele was used as a reference category for the estimates of case-only odds ratios and 95% confidence intervals. In a planned subgroup analysis, we limited our case-only study group to individuals exposed to COX-2 inhibitors and their matched unexposed comparison group. P-values were not corrected for multiple testing.

3.2.4 Results

We identified 115 NSAID exposed MI patients treated with rofecoxib (n=43), celecoxib (n=49), or another NSAID (n=23) within 10 days prior to hospital admission and compared them to 345 unexposed MI patients matched for age (+/-10y), sex and hospital center. Other NSAIDs included ibuprofen (n=7), naproxen (n=7), diclofenac (n=4), mesalamine (n=2), floctafenine (n=1), meloxicam (n=1) and high dose acetylsalicylic acid (n=1). The baseline characteristics of all patients are summarized in Table 3.2. The study population was typical of most acute coronary syndrome (ACS) reports with a mean age of 65 years, a male predominance and a high prevalence of conventional risk factors. Baseline characteristics were comparable between exposed and unexposed subjects; however, exposed patients had a slightly higher mean BMI, and were more likely to have diabetes and arterial hypertension. Treatment with regular or low dose aspirin (≤325mg) was recorded separately and was similar among exposed (52.2%) and unexposed (48.7%) patients.

Of 115 selected SNPS, 105 were successfully genotyped in at least 92.8% of patients. We observed statistically significant gene-drug interactions between NSAID exposure and 14 SNPs. The genotype counts and minor allele frequencies for these SNPs are shown in Table 3.3.

In the primary analysis of all individuals exposed to any NSAID, including COX-2 inhibitors, 12 SNPs significantly interacted with NSAID exposure (Table 3.4). The strongest p-value was observed between the homozygous minor allele of a SNP in the CRP gene and NSAID exposure (rs1205: OR=3.1; 95% confidence interval [CI], 1.5-6.2; P=0.002). However, the

strongest case-only odds ratios were observed between the homozygous minor alleles of two PTGS1 SNPs (rs10306135: OR=6.9; 95% CI, 1.4 – 35.4; P=0.02 and rs12353214: OR=4.6; 95% CI, 1.1 – 19.1; P=0.04). Significant associations were also observed with the heterozygotes of a SNP in PTGS2, the heterozygotes of four SNPs in the MMP1 gene, the homozygote minor allele of a SNP in the angiotensinogen (AGT) gene, the heterozygous and homozygous minor alleles of a variant in the Chr9p21.3 region, the heterozygotes of a SNPs in the ESR1 gene and the heterozygote of a SNP in the Klotho (KL) gene (Table 3.4).

To test for SNPs associated with the use of selective COX-2 inhibitors, the study group was limited to subjects exposed to rofecoxib (n=43) or celecoxib (n=49) and the matched unexposed subjects (n=276). In this subgroup analysis, the association between NSAID exposure and genotype strengthened for four SNPs (Table 3.4). Using the homozygous major allele as a reference, the homozygous minor allele yielded statistically significant case-only odds ratios for a SNP in the C-Reactive Protein (CRP) gene (rs1205: OR=3.6; 95% CI, 1.6 - 7.9; P=0.001) as well as two SNPs in the PTGS1 (COX-1) gene (rs10306135: OR=6.9; 95% CI, 1.4 – 35.7; P=0.02 and rs12353214: OR=6.9; 95% CI, 1.3 – 35.6; P=0.02). Within the Klotho gene, the heterozygote of one SNP (rs211247) yielded a statistically significant case-only odds ratio of 2.3 (95% CI, 1.4-4.0; P=0.002). In addition, one SNP in PTGS1 and one SNP in PTGS2 showed significant gene-drug interactions in the COX-2 subgroup analysis but were not significant in the primary analysis.

In a post-hoc analysis, significant SNP associations identified in the primary analysis were tested under the assumption of a recessive genetic model. In this analysis, 3 SNPs within PTGS1, one SNP in the chromosome 9p21.3 region, one SNP in CRP and one SNP in AGT were significantly associated with NSAID exposure (Table 3.5).

3.2.5 Discussion

Our findings show several interactions between SNPs and exposure to NSAIDs among acute coronary syndrome patients. We observed strong gene-drug

associations with two SNPS in the PTGS1 gene (rs10306135 and rs12353214), one in the CRP gene (rs1205) and one in the Klotho gene (rs211247) that were further strengthened in our COX-2 subgroup analysis. Furthermore, we found that variants of the chromosome 9 region, previously shown to be associated with coronary heart disease, interacted with exposure to NSAIDs to further increase the risk of coronary instability.

The two significant PTGS1 gene SNPs are located in intron 2 (rs10306135) and the 3' flanking region (rs12353214) of the gene. Several functional genetic variants within the PTGS1 gene have been shown to decrease arachidonic acid metabolism in vitro.[9] Furthermore there is evidence that some of these variants may significantly increase COX-1 sensitivity to the NSAID, indomethacin.[9] The PTGS1 gene is constitutively expressed in many cells throughout the body including platelets, gastrointestinal mucosa and the kidneys and has been associated with maintaining normal homeostasis in the heart.[19] Therefore, it is plausible that genetic variants within PTGS1 could make individuals more sensitive to the changes in COX-1 and COX-2 expression induced by NSAIDs or COX-2 inhibitors.

A strong interaction was also observed with a SNP (rs1205) in the C-Reactive Protein (CRP) gene. This protein is a known inflammatory marker and genetic variants within the gene have been associated with atherosclerosis, metabolic syndrome and coronary artery disease.[20, 21] Furthermore**,** several studies have shown that genetic variation within the CRP gene is associated with circulating CRP concentration.[20, 22] In particular, the minor allele of SNP rs1205, located in exon 2 of the CRP gene has been linked to decreased levels of serum CRP in several populations, as well as the development of systemic lupus erythematosus and decreased cardiovascular mortality.[23, 24] While previous research has focused on increased CRP levels as a risk factor for cardiovascular events, this study suggests that the minor allele of rs1205 (previously shown to be associated with low basal CRP levels [20, 23]) may interact with NSAIDs to increase cardiovascular risk. Given the association of rs1205 with lupus we must be careful to consider the possibility that this variant may be a predictor of NSAID

use thus violating the assumption of independence between genotype and exposure. However, only one patient in our study group reported having lupus and they were not being treated with NSAIDs.

Evidence of a gene-drug interaction was also observed with a tagging SNP (rs211247) in the 5' region of the Klotho gene (KL). To date, investigations of the KL gene have generated evidence of a role in the aging process as well as vascular function, hypertension and atherosclerotic disease.[25] Therefore, genetic variants of the Klotho gene may make an individual more susceptible to cardiovascular disease and it is possible that NSAIDs and COX-2 inhibitors may accelerate this process.

Finally, three recent independent genetic investigations have found that a region in chromosome 9 (9p21) is associated with coronary heart disease and myocardial infarction[26-28]. In the McPherson et al. study the reported hazard ratios for coronary heart disease were small but significant ranging from 1.18 to 1.29 for homozygote carriers of the major allele.[27] We investigated rs10757274 and found an odds ratio of 2.31 (95% CI, 1.2-4.5; P=0.01; GG vs AA, analysis not shown) suggesting that an interaction between the major allele and NSAIDs may further increase cardiovascular risk.

This case-only study has several limitations. First of all, it relies on the assumption of independence of genotype and exposure. Although we made every effort to limit the possibility of violating this assumption, we must consider this as a source of bias that may have resulted in significant findings. Secondly, some misclassification may have introduced bias into our results because some NSAIDs are available over the counter, and some patients taking NSAIDs on an as needed basis may have been recorded as unexposed. Finally, our study is limited by its modest sample size due to the barriers in collecting genetic samples and the cost of genotyping. However, our study provides justification for further investigation of several genes that may be linked to the cardiovascular risk of NSAIDs. Future case-control studies are necessary to confirm our assumption of independence of genotype and exposure, verify the interactions, and identify the individual risk of genotype alone and exposure alone.

In conclusion, our study provides evidence of gene-drug interactions influencing the cardiovascular outcomes of users of NSAIDs and selective COX-2 inhibitors. The strongest gene-drug associations observed within the COX-1, CRP and Klotho genes are candidates for further investigation. These findings suggest that genetic susceptibility may contribute to coronary instability in some users of this class of drugs.

Table 3.1 Candidate SNPs selected by literature review.

Candidate SNP	Gene or Region	Gene Symbol	References
rs943580	Angiotensinogen	AGT	[29, 30]
rs699	Angiotensinogen	AGT	[29, 30]
rs429358*	Apolipoprotein E	ApoE	[31]
rs7412*	Apolipoprotein E	ApoE	[31]
rs10757274	Chromosome 9	Chr9p21.3	[26, 27]
rs2383206	Chromosome 9	Chr9p21.3	[26, 27]
rs1205	C-Reactive Protein	CRP	[20-24]
rs9369217	Endothelin-1	EDN1	[32]
rs9380973	Endothelin-1	EDN1	[32]
rs5370	Endothelin-1	EDN1	[32]
rs3853248	Estrogen Receptor 1	ESR1	[33, 34]
rs11155814	Estrogen Receptor 1	ESR1	[33, 34]
rs7154455	Estrogen Receptor 2	ESR2	[33, 34]
rs3020450	Estrogen Receptor 2	ESR2	[33, 34]
rs543810	Interleukin-18	IL18	[35]
rs360722	Interleukin-18	IL18	[35]
rs211247	Klotho	KL	[25, 36]
rs854542	Paraoxonase-1	PON1	[37]
rs1805018	Phospholipase A2, group VII	PLA2G7	[38]
rs3219177	Resistin	RETN	[39]
rs13306848*	Thrombomodulin	THBD	[40]

*These SNPs were not successfully genotyped due to genotyping failure or error.

Table 3.2 Baseline characteristics of case subjects in the RISCA cohort

	NSAID Exposed (N=115)	NSAID Unexposed (N=345)
Mean Age - yr	64.5	64.7
Sex - no. (%)		
Male	79 (68.7%)	237 (68.7%)
Female	36 (31.3%)	108 (31.3%)
Admission Criteria - no. (%)		
Myocardial Infarction	70 (60.9%)	214 (62%)
Unstable Angina	45 (39.1%)	131 (38%)
Mean BMI	27.7	26.9
Smoking		
Current	25 (21.7%)	101 (29.3%)
Past	67 (58.3%)	162 (47.0%)
Never	23 (20.0%)	82 (23.8%)
Comorbidity - no. (%)		
Diabetes	27 (23.5%)	66 (19.1%)
Hypercholesterolemia	71 (61.7%)	220 (63.8%)
Arterial Hypertension	76 (66.1%)	179 (52.2%)
Prior Myocardial Infarction	34 (29.6%)	102 (29.6%)

Table 3.3 Genotype counts and minor allele frequencies of significant SNPs

Gene and SNP ID	Exposed				Unexposed			
	AA	Aa	aa	MAF	AA	Aa	aa	MAF
PTGS1								
rs10306135	79	27	6	0.17	234	95	3	0.15
rs12353214	97	10	5	0.09	284	45	3	0.08
rs2282169	68	37	7	0.23	207	115	8	0.20
PTGS2								
rs4648276	73	37	0	0.17	240	76	7	0.14
rs20417	66	44	2	0.21	226	98	8	0.17
MMP1								
rs7945189	94	13	3	0.09	249	76	3	0.13
rs5854	50	41	20	0.36	125	159	46	0.38
rs2071230	89	21	0	0.10	287	35	1	0.06
rs475007	30	67	15	0.43	116	151	65	0.42
AGT								
rs943580	43	53	15	0.37	115	142	75	0.44
Chr9p21.3								
rs10757274	45	51	16	0.37	93	174	63	0.45
CRP								
rs1205	40	54	18	0.40	162	147	23	0.29
ESR1								
rs3853248	92	16	3	0.10	246	82	2	0.13
KL								
rs211247	65	43	5	0.23	232	92	8	0.16

Table 3.4 Odds ratios for gene-drug interactions among cases

Gene and SNP ID	Genotype	All NSAID Users		Cox-2 Subgroup	
		Case Only Odds Ratio (95% CI)	P Value	Case Only Odds Ratio (95% CI)	P Value
PTGS1					
rs10306135†					
	AA	1.00		1.00	
	AT	0.88 (0.52-1.47)	0.619	0.88 (0.49-1.58)	0.678
	TT	6.91 (1.35-35.38)	0.020	6.94 (1.35-35.65)	0.020
rs12353214*†					
	CC	1.00		1.00	
	CT	0.65 (0.32-1.32)	0.236	0.77 (0.36-1.64)	0.499
	TT	4.55 (1.08-19.12)	0.039	6.87 (1.33-35.57)	0.022
rs2282169*†					
	GG	1.00		1.00	
	GC	0.99 (0.62-1.57	0.959	0.96 (0.57-1.61)	0.873
	CC	2.71 (0.88-8.35)	0.082	3.88 (1.09-13.82)	0.037
PTGS2					
rs4648276*†					
	TT	1.00		1.00	
	TC	1.66 (1.02-2.69)	0.041	1.92 (1.11-3.32)	0.020
	CC	-	-	-	-
rs20417*†					
	GG	1.00		1.00	
	GC	1.52 (0.96-2.40)	0.072	1.70 (1.02-2.85)	0.044
	CC	0.87 (0.18-4.15)	0.863	1.50 (0.29-7.87)	0.634
MMP1					
rs7945189*					
	CC	1.00		1.00	
	CT	0.47 (0.25-0.89)	0.021	0.47 (0.23-0.93)	0.031
	TT	2.19 (0.43-11.18)	0.346	3.42 (0.55-21.10)	0.186
rs5854*†					
	CC	1.00		1.00	
	CT	0.60 (0.36-0.97)	0.036	0.58 (0.34-1.01)	0.053
	TT	1.013 (0.55-1.87)	0.966	0.79 (0.39-1.61)	0.519
rs2071230†					
	AA	1.00		1.00	
	AG	1.88 (1.05-3.37)	0.034	1.74 (0.91-3.33)	0.097
	GG	-	-	-	-
rs475007*†					
	AA	1.00		1.00	
	AT	1.66 (1.01-2.71)	0.046	1.59 (0.92-2.73)	0.097
	TT	0.91 (0.46-1.80)	0.778	0.84 (0.39-1.78)	0.640
AGT					
rs943580*†					
	AA	1.00		1.00	
	AG	0.96 (0.61-1.51)	0.859	1.35 (0.80-2.29)	0.260
	GG	0.48 (0.24-0.96)	0.037	0.73 (0.34-1.60)	0.438
Chr9p21.3					
rs10757274‡					
	GG	1.00		1.00	
	GA	0.55 (0.32-0.92)	0.023	0.63 (0.35-1.12)	0.115
	AA	0.43 (0.22-0.84)	0.013	0.48 (0.23-1.01)	0.054
CRP					
rs1205					
	CC	1.00		1.00	
	CT	1.40 (0.89-2.22)	0.146	1.49 (0.88-2.50)	0.135
	TT	3.06 (1.51-6.16)	0.002	3.59 (1.64-7.86)	0.001
ESR1					
rs3853248*‡					
	TT	1.00		1.00	
	TC	0.50 (0.28-0.91)	0.023	0.52 (0.27-1.00)	0.050
	CC	3.70 (0.61-22.36)	0.154	7.86 (0.81-76.08)	0.075
KL					
rs211247					
	CC	1.00		1.00	
	CG	1.72 (1.08-2.75)	0.023	2.31 (1.37-3.90)	0.002
	GG	2.62 (0.76-9.08)	0.129	3.09 (0.78-12.20)	0.108

* Allele test was not significant
† Global genotype test was not significant.
‡ In complete linkage disequilibrium with another SNP tested

Table 3.5 Odds ratios for gene-drug interactions assuming a recessive model

Gene and SNP ID	Genotype	All NSAID Users		Cox-2 Subgroup	
		Case Only Odds Ratio (95% CI)	P Value	Case Only Odds Ratio (95% CI)	P Value
PTGS1					
rs10306135	TT	7.33 (1.46-36.88)	0.016	7.33 (1.46-36.88)	0.016
rs12353214	TT	4.77 (1.14-19.99)	0.033	7.11 (1.38-36.73)	0.019
rs2282169	CC	2.72 (0.89-8.26)	0.077	3.95 (1.13-13.78)	0.031
AGT					
rs943580	GG	0.49 (0.26-0.93)	0.029	0.60 (0.30 - 1.22)	0.158
Chr9p21.3					
rs10757274	GG	1.97 (1.22-3.19)	0.006	1.73 (1.01-2.97)	0.045
CRP					
rs1205	TT	2.54 (1.33-4.87)	0.005	2.94 (1.41-6.11)	0.004

Reference group is the homozygous major allele combined with the heterozygote with the exception of rs10757274 in which the reference group is the homozygous minor allele combined with the heterozygote.

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Chapter 4

Additional Results and Discussion

The primary results of this project are reported in the manuscript presented in Chapter 3 of this thesis. This chapter contains additional analyses and discussion that could not be included in the manuscript due to space limitations.

4.1 Power Calculations

The calculation of the sample size for case-only designs is a complex function of "the prevalence of exposure (e) and genotype (g), the relative risk for exposure alone (Re) and for genotype alone (Rg) and the effect of the gene-environment interaction (Ri), the type I error (alpha), and the type II error (beta)".[1] The power of this study is difficult to estimate because it varies depending on the allele frequency of each genotype and the genetic model being tested. However, power calculations were conducted for the four significant variants in the PTGS1, CRP and KL genes that were maintained in the COX-2 subgroup analysis as well as one of the Chr9p21.3 variants. All power calculations were conducted with Quanto Software, Version 1.2.3 (http://hydra.usc.edu/gxe/). The prevalence of the environmental exposure (NSAIDs) was assumed to be 25% and the baseline risk of the outcome (MI or UA) was assumed to be 0.0014.[2, 3] Two sets of calculations were performed, one assuming the risk of exposure alone and the risk of genotype alone were both equal to 1, and one assuming the individual risks were both equal to 1.2 (Table 4.1). Based on these calculations this study was powered over 80% for all five SNPs if a dominant or log-additive model is assumed. This study was less powered for the recessive genetic model with estimated powers ranging from 49.7% - 99.9% depending on the SNP. It is important to note that these power calculations are provided to give a general idea of the power of this case-only study to detect gene-environment interactions. These calculations do not take into account the matching technique used in this study. However, it is assumed that matching does not reduce statistical power and these estimates provide, at least, a conservative estimate of power.

These results affirm that the power of genetic studies fluctuates considerably depending on the allele frequency and the assumed genetic model.

Given the complexity and assumptions that go into power calculations for genetic studies, these estimates should not be relied on to affirm that a study is of an appropriate sample size. In this study, further investigation to confirm both positive and negative findings are warranted.

4.2 Assessing Hardy Weinberg Equilibrium

The law of Hardy-Weinberg Equilibrium (HWE) is "the principle that both gene and genotype frequencies will remain in equilibrium in an infinitely large population in the absence of mutation, migration, selection and nonrandom mating. If p is the frequency of one allele and q is the frequency of another and p + q = 1, then p^2 is the frequency of homozygotes for the allele, q^2 is the frequency of homozygotes for the other allele, and 2pq is the frequency of heterozgyotes".[4]

The merit of testing HWE in case-control studies is still under debate; therefore, it is still unclear what value they may hold in case-only studies. While some feel it is a valuable tool to detect genotyping errors, or as a method of detecting associations; others suggest that relying on this test may in fact be harmful to study interpretation.[5] We used the test for HWE in our exposed and unexposed cases simply as a method of further exploring our data (Appendix 3). It was interesting to note that in one of the SNPS in PTGS1 (rs12353214) shown to have a significant interaction with NSAIDs, there was a strong violation of HWE in exposed cases. According to the law of HWE, with a minor allele frequency of 0.08 we would expect only 1 out of the 112 exposed individuals tested to be a homozygote for the minor allele; however, we observed 5. In case-control studies, some people suggest this deviation from HWE can indicate the marker is associated with the disease of interest.[6] In this case-only study deviation from HWE suggests that at this marker exposed individuals are not randomly selected from the population; therefore, one of the alleles may be associated with the exposure. However, this analysis was simply performed to explore our data, was given little weight and was not used in the primary analysis.

4.3 Complete Genotyping and Regression Results for all SNPs

The complete genotyping results of all 115 SNPs investigated in our study could not be presented in the manuscript due to space limitations. The complete genotype frequencies and minor allele frequencies are presented in Appendix 3. Of 115 SNPs, 10 SNPs failed at the genotyping stage and are shaded in grey in the table. Those SNPs which showed a significant association either in the primary analysis or COX-2 subgroup analysis are highlighted in bold. The complete results of the conditional logistic regression using the major allele as a reference are presented in Appendix 3. These results are consistent with those presented in the manuscript.

In addition to the genotype test presented in the manuscript, we conducted a global genotype test to compare the distributions of genotypes between the exposed and unexposed groups as well as the allele count test to compare the frequencies of the major allele versus the minor allele between the exposed and unexposed groups. The results of these analyses are presented in Appendix 3.

For the global genotype test, a significant difference in genotype distributions between exposed and unexposed groups was observed in two SNPs in the Chr9p21.3 region (rs10757274 and rs2383206), one in the CRP gene (rs1205), two SNPs in the ESR1 gene (rs11155814 and rs3853248), one SNP in the Klotho gene (rs211247), one in the MMP1 gene (rs7945189) and one SNP in the PTGS1 gene (rs10306135). All of these significant associations were also detected with the genotype test presented in the manuscript.

In the allele count test, each patient is counted twice once for each allele inherited. Therefore, those who are homozygous (AA or aa) are counted twice into the appropriate allele group and those that are heterozygous are counted once in the major allele group (A) and once in the minor allele group (a). This test looks for associations with a particular allele rather than with the genotype. It is important to note that this test relies on the assumption that the population of cases and controls combined is in HWE.[7] Furthermore, it assumes that the addition of one allele contributes to the risk estimate and the addition of two alleles results in a multiplicative effect on risk (i.e the risk of the allele squared).

Therefore, this test is not robust to violations of HWE and may not be robust to recessive models or rare alleles. However, we still conducted the allele test to further explore our data. In this analysis a significant difference in allele distributions between exposed and unexposed groups was observed in two SNPs in the Chr9p21.3 region (rs10757274 and rs2383206), one in the CRP gene (rs1205), one SNP in the Klotho gene (rs211247) and one in the MMP1 gene (rs2071230). All of these significant associations were also detected in the genotype test presented in the manuscript.

4.4 COX-2 Subgroup Analysis

As discussed in the manuscript, we conducted a planned subgroup analysis consisting of all exposed cases who reported treatment with the COX-2 inhibitors, rofecoxib and celecoxib, and their corresponding matched unexposed cases. The complete results of this subgroup analysis for all 115 SNPs tested are presented in Appendix 3. The significant results from this subgroup analysis are presented and discussed in the manuscript.

4.5 Assessment of Significant Gene-Drug Interactions

Of 115 SNPS investigated, significant interactions were observed among 16 SNPs in the primary analysis and 7 SNPs in the COX-2 subgroup analysis. These include 3 SNPs in the PTGS1 gene, 2 SNPs in the PTGS2 gene, 4 SNPs in the MMP1 gene, 1 SNP in the AGT gene, 2 SNPs in complete linkage disequilibrium from the Chr9p21.3 region, 1 SNP in the CRP gene, 2 SNPs in complete linkage disequilibrium in the ESR1 gene and 1 SNP in the KL gene. All of these SNPs are synonymous and do not appear to code for functional variants; however, they may act as markers for functional variants within the gene.

As with any exploratory study, the interpretation of these significant results must be cautious. In this study, significant associations may be due to one of several scenarios. First of all, these associations may simply be spurious findings. In the context of multiple testing, we always expect to find some significant findings completely by chance. However, at a p-value of 0.05 we

would expect 5% or approximately 6 significant findings when testing 115 SNPs. Because we found 12 independent interactions in the primary analysis plus 2 additional independent interactions in the COX-2 subgroup analysis, it is not unreasonable to suspect that some of these are true positive findings.

Secondly, although we made every effort to limit the possibility of violating the assumption of independence between genotype and exposure, we must also consider this as a source of bias that may have resulted in significant findings. Given that some of the genes that we studied have roles in both coronary heart disease as well as other inflammatory disorders, there is a possibility that an associations may be indicative of a genotype predictive of NSAID use rather than a gene-drug interaction. This possibility is simply a limitation of our study design which can be addressed by further investigations aimed at replicating these findings.

Finally, some of the significant associations may be true gene-drug interactions. In order to distinguish between associations that are suggestive of true rather than spurious findings, we assessed the strength of each association on several criteria. First of all, we were particularly interested in SNPs that showed a significant association in the primary analysis and remained significant when the analysis was limited to the COX-2 subgroup. Secondly, we were interested in associations with strong odds ratios (>3) as our study is more powered to detect strong associations. Thirdly, we were interested in strong odds ratios which would be more robust had we adjusted for multiple testing. Finally, we considered the results of the global genotype test and allele count test. Table 4.2 shows the assessments of these criteria. Based on our analysis, rs1205 of the CRP gene, rs10306135 of the PTGS1 gene, and rs211247 of the Klotho gene are the strongest candidates for true gene-drug interactions.

4.6 Further Discussion of CRP, Klotho and PTGS1

The gene-drug interaction between a SNP in the CRP gene (rs1205) and NSAIDs and COX-2 inhibitors is somewhat difficult to interpret. As discussed in the manuscript, this protein is a known inflammatory marker and associations

have been found with atherosclerosis, ischemic stroke, metabolic syndrome and coronary artery disease.[8] Typically, high CRP levels are associated with an increased risk for cardiovascular events and the presence of inflammatory disease.[9, 10] However, among patients admitted for an acute coronary syndrome we found that patients homozygous for the minor allele of rs1205 (previously shown to be associated with low basal CRP levels[11, 12]) were significantly more likely to be exposed to NSAIDs. There are several possible interpretations of this result, including the following which are purely speculation: basal serum CRP may have some cardioprotective function thus making patients with genetically mediated low CRP levels more susceptible to cardiovascular events upon use of NSAIDS or COX-2 inhibitors; or low CRP is predictive of NSAID use thus violating our assumption of independence of genotype and exposure; or the result is simply a spurious finding. However, this interaction produced strong odds ratio estimates (NSAID: 3.06; COX-2: 3.59) and the strongest p-values (NSAID: P=0.002; COX-2: P=0.001) of all 115 SNPs investigated. Furthermore, c-reactive protein has been implicated in cardiovascular function. Therefore, this intriguing result should be followed up in further investigations.

The significant gene-drug interaction observed a SNP (rs211247) in the KL gene is also difficult to interpret. This SNP was selected as a tagging SNP for the 5' region of the gene. The flanking regions of genes are of particular interest because it is believed that genetic variants within these regions are more likely to affect the regulation of the gene. Although, there are no previous studies on rs211247 to suggest how genetic variation at this location may affect the gene, some studies suggest variants within the promoter region may be linked to hypertension.[13] Nevertheless, it is possible that genetic variants within the KL gene may make an individual more susceptible to cardiovascular disease and the use of NSAIDs and COX-2 inhibitors may accelerate this process. Further investigation into the role of the Klotho gene in acute coronary syndromes and into a possible gene-drug interaction with NSAIDs is justified.

Finally, significant gene-drug interactions were observed for two SNPs in the PTGS1 gene and these interactions were strengthened in the COX-2 subgroup.

Furthermore, another SNP in the PTGS1 gene was not significant in the primary analysis but was significant in the COX-2 subgroup analysis. The odds ratios of these observations were strong (Table 3.4). These results are particularly interesting because of the possibility that these SNPs may be markers for a functional variant. At this time, several functional genetic variants within the PTGS1 gene have been identified, and have been shown to decrease arachidonic acid metabolism in vitro.[14] Furthermore, there is evidence that some of these variants may significantly increase COX-1 sensitivity to the NSAID, indomethacin.[14] Of course, these results are also difficult to interpret. If we believe that COX-2 inhibitors increase cardiovascular risk because they decrease the presence of antithrombotic prostacyclin creating an imbalance with prothrombotic thromboxane, we might expect a beneficial effect of PTGS1 genetic variants that decrease basal COX-1 metabolic activity because they would also decrease the metabolism of prothrombotic thromboxane. However, if the PTGS1 SNPs we tested are markers for these functional genetic variants which decrease COX-1 activity, they appear to actually be harmful in the presence of NSAIDs and COX-2 inhibitors.

Although this seems paradoxical, we must consider that cardiovascular risk induced by COX-2 inhibitors may not be as simple as an imbalance between prostacyclin and thrombaxane. Indeed, the mechanism could be far more complex. Undoubtedly, there are many uncertainties as to the true biological mechanism of increased cardiovascular risk among COX-2 users. However, the results of this study show that it is very plausible that genetic variation, particularly in COX-1, may play an important role.

Table 4.1 Power calculations using Quanto

Significance level: 0.05, two sided
Assumed Prevalence of Environmental Exposure=0.25
Assumed Baseline Risk of MI=0.0014
Assumed Main Effect Genotype=1.00; Main Effect Environment=1.00
Cases=460

Power Estimate (Assume Rg=1, Re=1)						
Gene	**SNP**	**Minor Allele Frequency**	**Relative Risk**	**Recessive**	**Dominant**	**Log Additive**
PTGS1	rs10306135	0.17	6.91	0.999	0.999	0.999
PTGS1	rs12353214	0.09	4.55	0.497	0.999	0.999
Chr9p21.3	rs10757274	0.37	0.43	0.585	0.931	0.987
CRP	rs1205	0.40	3.06	0.998	0.999	1.000
KL	rs211247	0.23	2.62	0.735	0.998	1.000

Power Estimate (Assume Rg=1.2, Re=1.2)						
Gene	**SNP**	**Minor Allele Frequency**	**Relative Risk**	**Recessive**	**Dominant**	**Log Additive**
PTGS1	rs10306135	0.17	6.91	0.999	0.999	0.999
PTGS1	rs12353214	0.09	4.55	0.594	0.999	0.999
Chr9p21.3	rs10757274	0.37	0.43	0.687	0.949	0.995
CRP	rs1205	0.40	3.06	0.993	0.998	0.999
KL	rs211247	0.23	2.62	0.823	0.999	0.999

Table 4.2 Assessment of Significant Interactions

Gene	SNP	Significant Genotype Test (Major Allele as Reference)		Odds Ratio >3	P-Value <0.01	Significant Global Genotype Test	Significant Allele Count Test
		All NSAID Users	COX-2 Subgroup				
AGT	rs943580	Yes	No	No	No	No	No
Chr9p21.3*	rs10757274	Yes	No	No	No	Yes	Yes
CRP	rs1205	Yes	Yes	Yes	Yes	Yes	Yes
ESR1*	rs3853248	Yes	No	No	No	Yes	No
KL	rs211247	Yes	Yes	No	No	Yes	Yes
MMP1	rs7945189	Yes	Yes	No	No	Yes	No
MMP1	rs2071230	Yes	No	No	No	No	Yes
MMP1	rs5854	Yes	No	No	No	No	No
MMP1	rs475007	Yes	No	No	No	No	No
PTGS1	rs10306135	Yes	Yes	Yes	No	No	Yes
PTGS1	rs12353214	Yes	Yes	Yes	No	No	No
PTGS1	rs2282169	No	Yes	No	No	No	No
PTGS2	rs4648276	Yes	No	No	No	No	No
PTGS2	rs20417	No	Yes	No	No	No	No

*These SNPs were in complete linkage disequilibrium with another SNP tested

4.7 References

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Chapter 5

Conclusions

5 Conclusions

The increase in cardiovascular risk observed among users of NSAIDs and COX-2 inhibitors is the subject of ongoing investigation. This thesis investigated the role of genetic polymorphisms, and found evidence of several gene-drug interactions that may explain why some users of NSAIDs or COX-2 inhibitors experience adverse cardiovascular events.

Utilizing the case-only study design, we found significant gene-drug interactions between 12 independent SNPs in the primary analysis plus 2 additional interactions in the COX-2 subgroup. Based on several criteria we felt the strongest candidates for further investigation were those SNPs located in the PTGS1 (COX-1) gene, the CRP gene and the Klotho gene. Given the large number of interactions, the strength of the odds ratios, and the strength of p-values, this study provides justification for further investigation of the role of these genes in cardiovascular events among NSAID users. The easiest way to potentially replicate these findings would be to conduct a second case-only study recruiting patients with acute coronary syndrome. Furthermore, a future case-control study would be helpful in confirming our assumption of independence of genotype and exposure, verifying the interactions as well as identifying the individual risk of genotype alone and exposure alone.

The study of gene-environment interactions is relatively new. However, as access to the human genome and genetic material increase, these types of genetic studies will be more accessible to academic investigators. Continued research in this area will refine methodology and provide valuable insight into the role of genetics in the individual response to pharmacotherapy. In the case of NSAIDs and cardiovascular events, further study of these potential gene-drug interactions may provide some additional insight into the mechanism whereby previously stable coronary atherosclerosis becomes unstable leading to clinical events. Ultimately, a complete understanding of genetic susceptibility to adverse events may allow physicians to one day tailor prescribed drugs to each individual patient.

Appendices

Appendix 1: Genetic Epidemiology Glossary

-765G>C: genetic notation indicating a polymorphism 765 base pairs in front of the start codon (i.e in the promoter region) with the major allele G and the minor allele C.

allele: each of the different states found at a polymorphic site. Different alleles and their combinations may result in different phenotypes.[1]

candidate gene: a known gene suspected to be associated with the disease of interest on the basis of the biological function of its protein[2]

case-only design: approach to screen gene-environment interactions under the assumption of independence between exposure and genotype in the population. This design does not require control subjects. Therefore, sample sizes will be less than half than those required in case-control studies and the estimated odds ratios will not suffer from potential biases related to control selection.[3]

chromosome: linear DNA molecule that constitutes the basic physical block of heredity. Chromosomes in humans come in pairs; each member of a pair is inherited from one of the parents. Humans carry 23 pairs of chromosomes (22 pairs of autosomes and two sex chromosomes)[1]

constitutive: continuous expression of a gene resulting in ongoing production of its protein

CYP2C9*1/*3: notation for the CYP2C9 gene indicating an individual carrying alleles 1 and 3

DNA (deoxyribonucleic acid): macromolecule that constitutes the basis of heredity. It is a double helix made up of four different types of subunits or nucleotides: adenine, guanine, cytosine, and thymine (or A, G,C, and T).[1]

exon: each of the segment s in a gene that are transcribed, and whose transcripts are spliced together to form the messenger RNA.[1]

gene: DNA segment that is transcribed into messenger RNA and translated into a protein, Genes comprise the exons that are actually translated plus the intervening introns.[1]

genetic polymorphism: Genome segment (locus), within or outside a gene, in which alternate forms (alleles) are present.[2]

genome: whole set of the DNA of a species. The human genome is made of 23 pairs of chromosomes plus mitochondrial DNA, for a total of over 3200 million base pairs.[1]

genotype: the genetic constitution of an organism, which is modulated by the environment before being expressed as a phenotype[2]

haplotype: set of allelic states found at neighbouring loci in a chromosome, as inherited from a parent. Haplotypes can be broken down by recombination. A haplotype share among unrelated individuals affected with a genetic disease may indicate that a gene causing the disease maps to that genomic region.[2]

Hardy-Weinberg Equilibrium: state in which the allele and genotype frequencies do not change from one generation to the next in a population. It requires random mating and the absence of selection, mutation, migration, and genetic drift. In Hardy-Weinberg equilibrium, allele and genotype frequencies are related through the Hardy-Weinberg law: for a locus with two alleles P, Q at frequencies p and q respectively, homozygotes for p are found at frequency p^2, homozygotes for q have a frequency of q^2, and heterozygotes are found at a frequency 2pq. Although conditions for Hardy-Weinberg equilibrium are seldom strictly met, genotype frequencies are usually consistent with the Hardy-Weinberg law.[2]

heterozygote: individual that carries two different alleles at the same site in the two homologous chromosomes of a given pair.[1]

homozygote: individual that carries two copies of the same alleles at the same site in the two homologous chromosomes of a given pair.[1]

inducible gene: a gene whose expression increases in response to an environmental signal (or inducer)

intron: each of the segments of a gene that are not transcribed into messenger RNA that are found between exons[1]

linkage disequilibrium: a condition in which alleles at two loci or genes are found together in a population at a greater frequency than that predicted simply by the product of their individual allele frequencies. Alleles at markers near disease causing genes tend to be in linkage disequilibrium in the affected individuals. This is particularly the case in isolated, homogenous populations, in which it can be assumed that most affected individuals carry the same mutation.[3]

locus: any given genome region[1]

marker: any neutral polymorphism used in linkage or association analysis[2]

messenger RNA (mRNA): any RNA molecule that results from the transcription of a particular gene. mRNA takes the genetic information from the cell nucleus to the cytoplasm, where it is translated into proteins in the ribosomes.[1]

phenotype: expressed traits or characteristics of an organism, regardless of whether or to what extent the traits are the result of genotype or environment, or of the interaction of both.[2]

single nucleotide polymorphism (SNP): a DNA variant that represents variation in a single base. A common SNP can be defined as a locus at which two SNP alleles are present, both at a frequency of 1% or more. Across the human genome there could be 10 million common SNPS.[4]

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This glossary was primarily extracted and adapted from a series of three glossaries published in the Journal of Epidemiology and Community Health[1-3]. Permissions for adaptation were received on October 29, 2007 (License Numbers, 1818330708263, 1818330852921, and 1818331108206)

Appendix 2: List of Acronyms and Abbreviations

AGT: angiotensinogen

APPROVe: Adenomatous Polyp Prevention on Vioxx

Chr9p21.3: chromosome 9, p arm of the chromosome, region 21.3

CK-MB: creatine kinase - MB

CLASS: Celecoxib Long-term Arthritis Safety Study

COX-1: cyclooxygenase-1

COX-2: cyclooxygenase-2

CRP : c-reactive protein

CYP2C9: cytochrome p450, family 2, subfamily C, polypeptide 9

EDN1: endothelin 1

ESR1: estrogen receptor 1

ESR2: estrogen receptor 2

GI: gastrointestinal

HWE: Hardy Weinberg equilibrium

IL18: interleukin-18

KL: klotho

MI: myocardial infarction

MMP1: matrix metalloproteinase 1

MMP3: matrix metalloproteinase 2

MMP9: matrix metalloproteinase 9

NSAID: non-steroidal anti-inflammatory drug

OR: odds ratio

PG: prostaglandin

PGH_2: prostaglandin H_2

PGI_2: prostaglandin I_2 (prostacyclin)

PLA2G7: phospholipase A2, group VII

PON1: paraoxonase 1

PTGIS: prostaglandin I_2 synthase

PTGS1: prostaglandin endoperoxide synthase 1

PTGS2: prostaglandin endoperoxide synthase 2

RETN: resistin

RISCA: Recurrence et Inflammation dans les Syndromes Coronariens Aigus

SNP: single nucleotide polymorphism

THBD: thrombomodulin

TXA_2: thromboxane A_2

UA: unstable angina

VIGOR: Vioxx GI Outcomes Research

Appendix 3: Complete Analysis of 115 SNPs

Hardy Weinberg Equilibrium Calculations in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Gene	Marker	Exposed Genotype Frequencies			HWE in Exposed	Unexposed Genotype Frequencies			HWE in Unexposed
		AA	Aa	aa	P-Value	AA	Aa	Aa	P-Value
AGT	rs699	40	57	15	0.5518	110	144	76	**0.0345**
AGT	rs943580	43	53	15	1.0000	115	142	75	**0.0191**
APOE	rs429358				-				-
APOE	rs7412				-				-
Chr9p21.3	rs10757274	44	49	17	0.6843	87	171	74	0.5846
Chr9p21.3	rs2383206	45	51	16	0.8399	93	174	63	0.2689
CRP	rs1205	40	54	18	1.0000	162	147	23	0.2303
CYP2C9	rs1799853				-				-
CYP2C9	rs1856908	47	48	16	0.5383	137	147	48	0.4087
CYP2C9	rs1934963	69	37	6	0.7815	205	110	15	1.0000
CYP2C9	rs1934967	68	40	4	0.7782	212	102	18	0.2421
CYP2C9	rs1934968	88	23	0	0.6012	264	59	4	0.7605
CYP2C9	rs2153628	72	36	5	0.7769	224	94	15	0.2071
CYP2C9	rs2298037	75	35	2	0.5183	217	107	8	0.2759
CYP2C9	rs2860968	87	24	1	1.0000	238	87	7	1.0000
CYP2C9	rs4918766	43	52	16	1.0000	119	162	51	0.8188
CYP2C9	rs9332197	104	9	0	1.0000	295	34	4	**0.0320**
CYP2C9	rs9332238	70	36	6	0.5820	205	111	12	0.6139
EDN1	rs5370	78	28	1	0.6875	221	89	16	0.0968
EDN1	rs9369217	90	22	1	1.0000	263	59	10	**0.0141**
EDN1	rs9380973				-				-
ESR1	rs11155814	92	16	3	0.0689	246	82	2	0.0916
ESR1	rs3853248	92	16	3	0.0689	246	82	2	0.0916
ESR2	rs3020450	47	56	10	0.2961	145	154	34	0.5375
ESR2	rs7154455	47	55	10	0.3951	146	153	33	0.5344
IL18	rs360722	90	22	1	1.0000	263	65	5	0.5876
IL18	rs543810	89	23	1	1.0000	263	65	5	0.5876
KL	rs211247	65	43	5	0.7924	232	92	8	1.0000
MMP1	rs10488	95	15	2	0.1700	298	32	1	0.5906
MMP1	rs1051121	110	3	0	1.0000	309	24	0	1.0000
MMP1	rs1144393	38	58	16	0.5547	122	154	55	0.6464

Hardy Weinberg Equilibrium Calculations in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Gene	Marker	Exposed Genotype Frequencies			HWE in Exposed	Unexposed Genotype Frequencies			HWE in Unexposed
		AA	Aa	aa	P-Value	AA	Aa	Aa	P-Value
MMP1	rs17293761	104	9	0	1.0000	296	36	1	1.0000
MMP1	rs17879165	111	1	0	1.0000	332	0	0	0.0000
MMP1	rs1799750	31	57	24	1.0000	99	157	76	0.3781
MMP1	rs2071230	89	21	0	0.5936	287	35	1	1.0000
MMP1	rs2071232	70	38	3	0.5575	225	93	10	0.8482
MMP1	rs2408489	68	33	12	**0.0227**	223	84	26	**0.0001**
MMP1	rs2408490	80	30	3	1.0000	225	98	10	1.0000
MMP1	rs3213460	83	28	1	0.6878	244	85	3	0.1665
MMP1	rs470221	72	35	5	0.7696	234	90	8	1.0000
MMP1	rs475007	30	67	15	**0.0335**	116	151	65	0.2171
MMP1	rs484915	31	56	23	0.8510	107	158	66	0.5779
MMP1	rs498186	35	56	22	1.0000	106	160	67	0.6570
MMP1	rs5031036	96	14	2	0.1402	300	32	1	0.5883
MMP1	rs514921	65	41	7	0.8103	174	128	31	0.2849
MMP1	rs5854	50	41	20	**0.0397**	125	159	46	0.7271
MMP1	rs7125062	54	48	10	1.0000	190	118	22	0.5521
MMP1	rs7945189	94	13	3	**0.0300**	249	76	3	0.4457
MMP3	rs3025058	36	52	24	0.5666	97	166	68	0.9119
MMP3	rs3025065	110	0	0	0.0000	331	1	0	1.0000
MMP3	rs3025066	90	20	0	0.5959	275	45	3	0.4325
MMP3	rs476762	91	20	2	0.3537	259	69	5	0.7945
MMP3	rs522616	78	27	7	0.0526	225	91	15	0.1431
MMP3	rs527832	92	20	0	0.5972	274	54	4	0.5084
MMP3	rs591058	34	51	25	0.5641	97	168	67	0.7411
MMP3	rs650108	69	32	11	**0.0352**	190	119	22	0.5552
MMP9	rs13040272	58	35	19	0.6992	115	155	62	0.4328
MMP9	rs13040572	44	52	14	1.0000	137	138	48	0.1855
MMP9	rs13925	77	33	3	1.0000	245	78	10	0.2707
MMP9	rs13969				-				-
MMP9	rs17576	44	54	14	0.8387	139	145	48	0.3420
MMP9	rs1805088	108	5	0	1.0000	313	20	0	1.0000

Hardy Weinberg Equilibrium Calculations in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Gene	Marker	Exposed Genotype Frequencies			HWE in Exposed	Unexposed Genotype Frequencies			HWE in Unexposed
		AA	Aa	aa	P-Value	AA	Aa	Aa	P-Value
MMP9	rs1805089	112	0	0	0.0000	332	0	0	0.0000
MMP9	rs2236416	76	32	4	0.7493	241	80	10	0.2849
MMP9	rs2250889	98	11	0	1.0000	290	33	1	1.0000
MMP9	rs2274756	76	32	4	0.7493	242	79	10	0.2773
MMP9	rs3787268	75	33	3	1.0000	209	105	18	0.3253
MMP9	rs3918242				-				-
MMP9	rs3918251	37	59	16	0.4332	135	144	50	0.2873
MMP9	rs3918254	113	0	0	0.0000	333	0	0	0.0000
MMP9	rs3918278	107	6	0	1.0000	314	19	0	1.0000
MMP9	rs8125581	112	0	0	0.0000	332	0	0	0.0000
MMP9	rs9509	107	5	0	1.0000	308	22	0	1.0000
PLA2G7	rs1805018	103	9	0	1.0000	304	28	0	1.0000
PON1	rs854542	66	41	5	0.7926	197	126	9	**0.0357**
PTGIS	rs476496	55	47	11	0.8262	174	141	18	0.1593
PTGIS	rs477627	69	37	6	0.7815	223	97	11	0.8534
PTGIS	rs495146	71	38	4	1.0000	210	110	13	0.8668
PTGIS	rs501908	94	14	2	0.1446	272	44	3	0.4240
PTGIS	rs508757	74	36	2	0.5179	214	105	12	1.0000
PTGIS	rs5602	30	57	23	0.8479	93	165	68	0.8237
PTGIS	rs5628	103	8	1	0.1893	297	35	0	1.0000
PTGIS	rs574113	46	49	15	0.8356	141	152	38	0.8084
PTGIS	rs6019902	75	33	4	0.7606	222	99	11	1.0000
PTGIS	rs6019910	99	13	0	1.0000	292	38	1	1.0000
PTGIS	rs6090996	78	30	4	0.5166	218	99	14	0.4822
PTGIS	rs927068	66	43	4	0.4299	187	126	19	0.7697
PTGS1	rs10306114	103	10	0	1.0000	294	38	1	1.0000
PTGS1	rs10306135	79	27	6	0.0974	234	95	3	**0.0541**
PTGS1	rs10306202	94	18	0	1.0000	280	49	2	1.0000
PTGS1	rs1213266	96	16	0	1.0000	269	60	0	0.0912
PTGS1	rs12353214	97	10	5	**0.0003**	284	45	3	0.4245
PTGS1	rs1236913	99	13	0	1.0000	283	48	0	0.3967

Hardy Weinberg Equilibrium Calculations in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Gene	Marker	Exposed Genotype Frequencies			HWE in Exposed	Unexposed Genotype Frequencies			HWE in Unexposed
		AA	Aa	aa	P-Value	AA	Aa	Aa	P-Value
PTGS1	rs2282169	68	37	7	0.5893	207	115	8	0.1174
PTGS1	rs3842787	101	10	0	1.0000	286	37	2	0.3655
PTGS1	rs4836885	76	33	3	1.0000	248	80	4	0.4805
PTGS1	rs5789	102	10	0	1.0000	316	15	0	1.0000
PTGS1	rs6478565	71	35	5	0.7710	225	98	7	0.4344
PTGS2	rs12042763	67	35	9	0.1891	190	117	19	0.8786
PTGS2	rs20417	66	44	2	0.0974	226	98	8	0.5677
PTGS2	rs20432				-				-
PTGS2	rs2066826				-				-
PTGS2	rs2206593	99	13	0	1.0000	290	40	2	0.6441
PTGS2	rs2745557	77	30	5	0.3388	240	82	9	0.5206
PTGS2	rs4648261	107	3	0	1.0000	314	17	0	1.0000
PTGS2	rs4648276	73	37	0	**0.0387**	240	76	7	0.6479
PTGS2	rs4648298				-				-
PTGS2	rs5273	112	0	0	0.0000	330	1	0	1.0000
PTGS2	rs5275	41	56	13	0.4186	139	130	48	0.0660
PTGS2	rs5277	82	27	3	0.7034	234	92	6	0.5315
PTGS2	rs689466	72	36	4	1.0000	210	111	10	0.3868
PTGS2	rs689470	103	9	1	0.2253	317	16	0	1.0000
RETN	rs3219177	62	45	4	0.3009	204	107	20	0.2643
Error (CYP2C9)	rs10579140				-				-
Error (THBD)	rs1330684	38	60	12	0.1561	122	157	50	1.0000

Genotype and Minor Allele Frequencies in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Marker Information							Exposed to NSAID				Unexposed to NSAID			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	AA	Aa	aa	Minor Allele Frequency	AA	Aa	aa	Minor Allele Frequency
AGT	rs699	T	C	chr1:228912417	43.33%	69	40	57	15	0.39	110	144	76	0.45
AGT	**rs943580**	**A**	**G**	**chr1:228903667**	**42.33%**	**34**	**43**	**53**	**15**	**0.37**	**115**	**142**	**75**	**0.44**
APOE	rs429358	T	C	chr19:50103781	1.98%	Failed								
APOE	rs7412	C	T	chr19:50103919	10.00%	Failed								
Chr9p21.3	**rs10757274**	**G**	**A**	**chr9:22086055**	**45.48%**	**1**	**44**	**49**	**17**	**0.38**	**87**	**171**	**74**	**0.48**
Chr9p21.3	**rs2383206**	**G**	**A**	**chr9:22105026**	**43.33%**	**49**	**45**	**51**	**16**	**0.37**	**93**	**174**	**63**	**0.45**
CRP	**rs1205**	**C**	**T**	**chr1:157948857**	**31.87%**	**3**	**40**	**54**	**18**	**0.40**	**162**	**147**	**23**	**0.29**
CYP2C9	rs1799853	C	T	chr10:96692037	1.03%	Failed								
CYP2C9	rs1856908	G	T	chr10:96722721	36.46%	10	47	48	16	0.36	137	147	48	0.37
CYP2C9	rs1934963	T	C	chr10:96724666	21.38%	44	69	37	6	0.22	205	110	15	0.21
CYP2C9	rs1934967	C	T	chr10:96731416	20.95%	11	68	40	4	0.21	212	102	18	0.21
CYP2C9	rs1934968	G	A	chr10:96731807	10.27%	12	88	23	0	0.10	264	59	4	0.10
CYP2C9	rs2153628	A	G	chr10:96713414	19.06%	83	72	36	5	0.20	224	94	15	0.19
CYP2C9	rs2298037	C	T	chr10:96736068	18.24%	15	75	35	2	0.17	217	107	8	0.19
CYP2C9	rs2860968	T	C	chr10:96703571	14.30%	16	87	24	1	0.12	238	87	7	0.15
CYP2C9	rs4918766	G	A	chr10:96701874	39.28%	24	43	52	16	0.38	119	162	51	0.40
CYP2C9	rs9332197	T	C	chr10:96730898	5.72%	104	104	9	0	0.04	295	34	4	0.06
CYP2C9	rs9332238	G	A	chr10:96738482	20.80%	105	70	36	6	0.21	205	111	12	0.21
EDN1	rs5370	G	T	chr6:12404241	17.44%	61	78	28	1	0.14	221	89	16	0.19
EDN1	rs9369217	C	T	chr6:12391748	11.57%	106	90	22	1	0.11	263	59	10	0.12
EDN1	rs9380973	T	C	chr6:12392375	0.00%	Failed								
ESR1	**rs11155814**	**A**	**G**	**chr6:152192877**	**12.24%**	**38**	**92**	**16**	**3**	**0.10**	**246**	**82**	**2**	**0.13**
ESR1	**rs3853248**	**T**	**C**	**chr6:152181579**	**12.24%**	**54**	**92**	**16**	**3**	**0.10**	**246**	**82**	**2**	**0.13**
ESR2	rs3020450	G	A	chr14:63838055	33.41%	86	47	56	10	0.34	145	154	34	0.33
ESR2	rs7154455	G	C	chr14:63806413	33.11%	102	47	55	10	0.33	146	153	33	0.33
IL18	rs360722	C	T	chr11:111531913	11.10%	88	90	22	1	0.11	263	65	5	0.11
IL18	rs543810	A	G	chr11:111514188	11.21%	98	89	23	1	0.11	263	65	5	0.11
KL	**rs211247**	**C**	**G**	**chr13:32480646**	**18.09%**	**82**	**65**	**43**	**5**	**0.23**	**232**	**92**	**8**	**0.16**
MMP1	rs10488	G	A	chr11:102173232	5.98%	37	95	15	2	0.08	298	32	1	0.05
MMP1	rs1051121	C	T	chr11:102171183	3.03%	74	110	3	0	0.01	309	24	0	0.04
MMP1	rs1144393	T	C	chr11:102174619	39.96%	39	38	58	16	0.40	122	154	55	0.40
MMP1	rs17293761	C	T	chr11:102164443	5.27%	78	104	9	0	0.04	296	36	1	0.06
MMP1	rs17879165	G	A	chr11:102166730	0.11%	6	111	1	0	0.00	332	0	0	0.00
MMP1	rs1799750	-	G	chr11:102175706	46.62%	7	31	57	24	0.47	99	157	76	0.47

Genotype and Minor Allele Frequencies in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Marker Information							Exposed to NSAID				Unexposed to NSAID			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	AA	Aa	aa	Minor Allele Frequency	AA	Aa	aa	Minor Allele Frequency
MMP1	**rs2071230**	**A**	**G**	**chr11:102166169**	**6.70%**	**13**	**89**	**21**	**0**	**0.10**	**287**	**35**	**1**	**0.06**
MMP1	rs2071232	T	C	chr11:102170879	17.88%	81	70	38	3	0.20	225	93	10	0.17
MMP1	rs2408489	C	A	chr11:102162896	21.64%	84	68	33	12	0.25	223	84	26	0.20
MMP1	rs2408490	C	T	chr11:102177763	17.27%	85	80	30	3	0.16	225	98	10	0.18
MMP1	rs3213460	G	A	chr11:102174092	13.63%	18	83	28	1	0.13	244	85	3	0.14
MMP1	rs470221	G	A	chr11:102170480	17.00%	21	72	35	5	0.20	234	90	8	0.16
MMP1	**rs475007**	**A**	**T**	**chr11:102174522**	**42.57%**	**22**	**30**	**67**	**15**	**0.43**	**116**	**151**	**65**	**0.42**
MMP1	rs484915	A	T	chr11:102178458	44.44%	57	31	56	23	0.46	107	158	66	0.44
MMP1	rs498186	A	C	chr11:102174855	44.17%	95	35	56	22	0.44	106	160	67	0.44
MMP1	rs5031036	A	G	chr11:102171374	5.84%	96	96	14	2	0.08	300	32	1	0.05
MMP1	rs514921	A	G	chr11:102174440	27.47%	97	65	41	7	0.24	174	128	31	0.29
MMP1	**rs5854**	**C**	**T**	**chr11:102166084**	**37.64%**	**63**	**50**	**41**	**20**	**0.36**	**125**	**159**	**46**	**0.38**
MMP1	rs7125062	T	C	chr11:102168713	26.02%	70	54	48	10	0.30	190	118	22	0.25
MMP1	**rs7945189**	**C**	**T**	**chr11:102165774**	**11.53%**	**71**	**94**	**13**	**3**	**0.09**	**249**	**76**	**3**	**0.13**
MMP3	rs3025058	-	T	chr11:102221162	45.37%	51	36	52	24	0.45	97	166	68	0.46
MMP3	rs3025065	A	G	chr11:102216192	0.11%	17	110	0	0	0.00	331	1	0	0.00
MMP3	rs3025066	A	G	chr11:102215693	8.20%	87	90	20	0	0.09	275	45	3	0.08
MMP3	rs476762	T	A	chr11:102215917	11.55%	93	91	20	2	0.11	259	69	5	0.12
MMP3	rs522616	A	G	chr11:102220258	18.28%	25	78	27	7	0.18	225	91	15	0.18
MMP3	rs527832	C	T	chr11:102207094	9.24%	28	92	20	0	0.09	274	54	4	0.09
MMP3	rs591058	T	C	chr11:102216548	45.59%	30	34	51	25	0.46	97	168	67	0.45
MMP3	rs650108	G	A	chr11:102213997	24.49%	67	69	32	11	0.24	190	119	22	0.25
MMP9	rs13040272	T	C	chr20:44066518	42.23%	4	58	35	19	0.33	115	155	62	0.42
MMP9	rs13040572	A	C	chr20:44072376	36.26%	76	44	52	14	0.36	137	138	48	0.36
MMP9	rs13925	G	A	chr20:44078372	15.36%	77	77	33	3	0.17	245	78	10	0.15
MMP9	rs13969	C	A	chr20:44076240	0.00%	Failed								
MMP9	rs17576	A	G	chr20:44073632	36.37%	5	44	54	14	0.37	139	145	48	0.36
MMP9	rs1805088	C	T	chr20:44071031	2.80%	79	108	5	0	0.02	313	20	0	0.03
MMP9	rs1805089	G	A	chr20:44072017	0.00%	9	112	0	0	0.00	332	0	0	0.00
MMP9	rs2236416	A	G	chr20:44073982	15.80%	45	76	32	4	0.18	241	80	10	0.15
MMP9	rs2250889	C	G	chr20:44075813	5.31%	46	98	11	0	0.05	290	33	1	0.05
MMP9	rs2274756	G	A	chr20:44076518	15.69%	47	76	32	4	0.18	242	79	10	0.15
MMP9	rs3787268	G	A	chr20:44075138	20.32%	89	75	33	3	0.18	209	105	18	0.21
MMP9	rs3918242	T	C	chr20:44069383	0.00%	Failed								

Genotype and Minor Allele Frequencies in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Marker Information							Exposed to NSAID				Unexposed to NSAID			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	AA	Aa	aa	Minor Allele Frequency	AA	Aa	aa	Minor Allele Frequency
MMP9	rs3918251	A	G	chr20:44072188	37.98%	55	37	59	16	0.41	135	144	50	0.37
MMP9	rs3918254	C	T	chr20:44073798	0.00%	90	113	0	0	0.00	333	0	0	0.00
MMP9	rs3918278	G	A	chr20:44069061	2.80%	91	107	6	0	0.03	314	19	0	0.03
MMP9	rs8125581	G	A	chr20:44072650	0.00%	32	112	0	0	0.00	332	0	0	0.00
MMP9	rs9509	T	C	chr20:44078560	3.06%	35	107	5	0	0.02	308	22	0	0.03
PLA2G7	rs1805018	T	C	chr6:46787262	4.17%	8	103	9	0	0.04	304	28	0	0.04
PON1	rs854542	A	G	chr7:94759427	21.96%	33	66	41	5	0.23	197	126	9	0.22
PTGIS	rs476496	A	G	chr20:47611191	27.58%	92	55	47	11	0.31	174	141	18	0.27
PTGIS	rs477627	C	T	chr20:47613465	18.96%	56	69	37	6	0.22	223	97	11	0.18
PTGIS	rs495146	C	T	chr20:47563735	20.40%	94	71	38	4	0.20	210	110	13	0.20
PTGIS	rs501908	A	G	chr20:47589174	7.93%	58	94	14	2	0.08	272	44	3	0.08
PTGIS	rs508757	A	G	chr20:47572378	19.08%	59	74	36	2	0.18	214	105	12	0.19
PTGIS	rs5602	T	C	chr20:47555385	46.33%	99	30	57	23	0.47	93	165	68	0.46
PTGIS	rs5628	C	T	chr20:47574089	5.07%	29	103	8	1	0.04	297	35	0	0.05
PTGIS	rs574113	A	G	chr20:47553590	34.81%	100	46	49	15	0.36	141	152	38	0.34
PTGIS	rs6019902	G	A	chr20:47611620	18.24%	31	75	33	4	0.18	222	99	11	0.18
PTGIS	rs6019910	C	T	chr20:47623094	5.98%	64	99	13	0	0.06	292	38	1	0.06
PTGIS	rs6090996	G	A	chr20:47567189	18.62%	65	78	30	4	0.17	218	99	14	0.19
PTGIS	rs927068	G	T	chr20:47611381	24.16%	103	66	43	4	0.23	187	126	19	0.25
PTGS1	rs10306114	A	G	chr9:124172343	5.61%	72	103	10	0	0.04	294	38	1	0.06
PTGS1	**rs10306135**	**A**	**T**	**chr9:124177516**	**15.77%**	**73**	**79**	**27**	**6**	**0.17**	**234**	**95**	**3**	**0.15**
PTGS1	rs10306202	G	A	chr9:124199342	8.01%	36	94	18	0	0.08	280	49	2	0.08
PTGS1	rs1213266	G	A	chr9:124176705	8.62%	40	96	16	0	0.07	269	60	0	0.09
PTGS1	**rs12353214**	**C**	**T**	**chr9:124201691**	**8.00%**	**75**	**97**	**10**	**5**	**0.09**	**284**	**45**	**3**	**0.08**
PTGS1	rs1236913	C	T	chr9:124173300	6.89%	41	99	13	0	0.06	283	48	0	0.07
PTGS1	rs2282169	G	C	chr9:124180517	20.59%	48	68	37	7	0.23	207	115	8	0.20
PTGS1	rs3842787	C	T	chr9:124173328	5.85%	53	101	10	0	0.05	286	37	2	0.06
PTGS1	rs4836885	T	C	chr9:124186190	14.30%	23	76	33	3	0.17	248	80	4	0.13
PTGS1	rs5789	C	A	chr9:124183794	2.82%	62	102	10	0	0.04	316	15	0	0.02
PTGS1	rs6478565	A	G	chr9:124188453	17.80%	66	71	35	5	0.20	225	98	7	0.17
PTGS2	rs12042763	G	T	chr1:184918499	23.80%	2	67	35	9	0.24	190	117	19	0.24
PTGS2	rs20417	G	C	chr1:184916944	18.24%	80	66	44	2	0.21	226	98	8	0.17
PTGS2	rs20432	T	G	chr1:184912946	0.00%	Failed								
PTGS2	rs2066826	G	A	chr1:184912550	14.19%	Failed								

Genotype and Minor Allele Frequencies in NSAID Exposed and Unexposed Cases
(AA=homozygous for the major allele, Aa=heterozygous, aa=homozygous for the minor allele)

Marker Information							Exposed to NSAID				Unexposed to NSAID			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	AA	Aa	aa	Minor Allele Frequency	AA	Aa	aa	Minor Allele Frequency
PTGS2	rs2206593	G	A	chr1:184909052	6.42%	14	99	13	0	0.06	290	40	2	0.07
PTGS2	rs2745557	G	A	chr1:184915844	15.80%	50	77	30	5	0.18	240	82	9	0.15
PTGS2	rs4648261	G	A	chr1:184915627	2.27%	19	107	3	0	0.01	314	17	0	0.03
PTGS2	**rs4648276**	**T**	**C**	**chr1:184912111**	**14.67%**	**20**	**73**	**37**	**0**	**0.17**	**240**	**76**	**7**	**0.14**
PTGS2	rs4648298	A	G	chr1:184908305	2.54%	Failed								
PTGS2	rs5273	T	C	chr1:184910391	0.11%	60	112	0	0	0.00	330	1	0	0.00
PTGS2	rs5275	T	C	chr1:184909681	36.07%	26	41	56	13	0.37	139	130	48	0.36
PTGS2	rs5277	G	C	chr1:184914820	15.43%	27	82	27	3	0.15	234	92	6	0.16
PTGS2	rs689466	A	G	chr1:184917374	19.75%	68	72	36	4	0.20	210	111	10	0.20
PTGS2	rs689470	C	T	chr1:184907681	3.03%	101	103	9	1	0.05	317	16	0	0.02
RETN	rs3219177	C	T	chr19:7640369	22.62%	52	62	45	4	0.24	204	107	20	0.22
Error (CYP2C9)	rs10579140	C	-	chr11:65899405	0.00%	Failed								
Error (THBD)	rs1330684	G	A	chr9:129619420	38.84%	42	38	60	12	0.38	122	157	50	0.39

Primary Analysis: Odds Ratio Estimates of Gene-NSAID Interaction

Marker Information							Case-Only Odds Ratios and P-Values (Major Allele as Reference)								
							AA	Genotype Aa				Genotype aa			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	Odds Ratio	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
AGT	rs699	T	C	chr1:228912417	43.33%	69	1.00	1.08	0.68	1.73	0.740	0.55	0.28	1.08	0.084
AGT	**rs943580**	**A**	**G**	**chr1:228903667**	**42.33%**	**34**	**1.00**	**0.96**	**0.61**	**1.51**	**0.859**	**0.48**	**0.24**	**0.96**	**0.037**
APOE	rs429358	T	C	chr19:50103781	1.98%	Failed									
APOE	rs7412	C	T	chr19:50103919	10.00%	Failed									
Chr9p21.3	**rs10757274**	**G**	**A**	**chr9:22086055**	**45.48%**	**1**	**1.00**	**0.55**	**0.32**	**0.92**	**0.023**	**0.43**	**0.22**	**0.84**	**0.013**
Chr9p21.3	**rs2383206**	**G**	**A**	**chr9:22105026**	**43.33%**	**49**	**1.00**	**0.56**	**0.34**	**0.92**	**0.021**	**0.48**	**0.25**	**0.93**	**0.030**
CRP	**rs1205**	**C**	**T**	**chr1:157948857**	**31.87%**	**3**	**1.00**	**1.40**	**0.89**	**2.22**	**0.146**	**3.06**	**1.52**	**6.16**	**0.002**
CYP2C9	rs1799853	C	T	chr10:96692037	1.03%	Failed									
CYP2C9	rs1856908	G	T	chr10:96722721	36.46%	10	1.00	0.95	0.60	1.48	0.807	1.02	0.53	1.95	0.957
CYP2C9	rs1934963	T	C	chr10:96724666	21.38%	44	1.00	0.99	0.62	1.59	0.962	1.10	0.42	2.88	0.845
CYP2C9	rs1934967	C	T	chr10:96731416	20.95%	11	1.00	1.26	0.79	2.03	0.338	0.86	0.28	2.67	0.793
CYP2C9	rs1934968	G	A	chr10:96731807	10.27%	12	1.00	1.11	0.63	1.96	0.715	-	-	-	-
CYP2C9	rs2153628	A	G	chr10:96713414	19.06%	83	1.00	1.19	0.75	1.90	0.458	1.08	0.38	3.03	0.887
CYP2C9	rs2298037	C	T	chr10:96736068	18.24%	15	1.00	0.93	0.60	1.44	0.738	0.83	0.16	4.23	0.818
CYP2C9	rs2860968	T	C	chr10:96703571	14.30%	16	1.00	0.75	0.45	1.26	0.276	0.36	0.04	2.97	0.345
CYP2C9	rs4918766	G	A	chr10:96701874	39.28%	24	1.00	0.85	0.52	1.39	0.521	0.84	0.44	1.63	0.609
CYP2C9	rs9332197	T	C	chr10:96730898	5.72%	104	1.00	0.71	0.32	1.59	0.406	-	-	-	-
CYP2C9	rs9332238	G	A	chr10:96738482	20.80%	105	1.00	0.95	0.59	1.54	0.840	1.46	0.53	4.01	0.467
EDN1	rs5370	G	T	chr6:12404241	17.44%	61	1.00	0.94	0.57	1.57	0.819	0.18	0.02	1.38	0.099
EDN1	rs9369217	C	T	chr6:12391748	11.57%	106	1.00	1.11	0.65	1.90	0.710	0.28	0.03	2.21	0.225
EDN1	rs9380973	T	C	chr6:12392375	0.00%	Failed									
ESR1	**rs11155814**	**A**	**G**	**chr6:152192877**	**12.24%**	**38**	**1.00**	**0.51**	**0.28**	**0.92**	**0.026**	**3.71**	**0.61**	**22.40**	**0.153**
ESR1	**rs3853248**	**T**	**C**	**chr6:152181579**	**12.24%**	**54**	**1.00**	**0.50**	**0.28**	**0.91**	**0.023**	**3.70**	**0.61**	**22.36**	**0.154**
ESR2	rs3020450	G	A	chr14:63838055	33.41%	86	1.00	1.10	0.71	1.71	0.671	0.90	0.42	1.95	0.786
ESR2	rs7154455	G	C	chr14:63806413	33.11%	102	1.00	1.09	0.70	1.70	0.715	0.92	0.42	2.00	0.834
IL18	rs360722	C	T	chr11:111531913	11.10%	88	1.00	0.96	0.55	1.66	0.876	0.55	0.06	4.76	0.591
IL18	rs543810	A	G	chr11:111514188	11.21%	98	1.00	1.01	0.59	1.74	0.959	0.56	0.07	4.79	0.594
KL	**rs211247**	**C**	**G**	**chr13:32480646**	**18.09%**	**82**	**1.00**	**1.72**	**1.08**	**2.75**	**0.023**	**2.62**	**0.76**	**9.08**	**0.129**
MMP1	rs10488	G	A	chr11:102173232	5.98%	37	1.00	1.40	0.73	2.66	0.311	6.00	0.54	66.14	0.144
MMP1	rs1051121	C	T	chr11:102171183	3.03%	74	1.00	0.34	0.10	1.17	0.087	-	-	-	-
MMP1	rs1144393	T	C	chr11:102174619	39.96%	39	1.00	1.16	0.71	1.88	0.559	0.89	0.46	1.72	0.732
MMP1	rs17293761	C	T	chr11:102164443	5.27%	78	1.00	0.75	0.35	1.62	0.463	-	-	-	-
MMP1	rs17879165	G	A	chr11:102166730	0.11%	6	1.00	-	-	-	-	-	-	-	-
MMP1	rs1799750	-	G	chr11:102175706	46.62%	7	1.00	1.16	0.70	1.92	0.575	1.01	0.54	1.88	0.978
MMP1	**rs2071230**	**A**	**G**	**chr11:102166169**	**6.70%**	**13**	**1.00**	**1.88**	**1.05**	**3.37**	**0.034**	**-**	**-**	**-**	**-**
MMP1	rs2071232	T	C	chr11:102170879	17.88%	81	1.00	1.45	0.89	2.36	0.140	1.05	0.27	4.00	0.947
MMP1	rs2408489	C	A	chr11:102162896	21.64%	84	1.00	1.37	0.83	2.28	0.224	1.56	0.74	3.30	0.244
MMP1	rs2408490	C	T	chr11:102177763	17.27%	85	1.00	0.83	0.51	1.36	0.461	0.93	0.25	3.48	0.912
MMP1	rs3213460	G	A	chr11:102174092	13.63%	18	1.00	0.98	0.59	1.62	0.934	0.80	0.08	7.84	0.849
MMP1	rs470221	G	A	chr11:102170480	17.00%	21	1.00	1.22	0.78	1.92	0.390	1.98	0.62	6.39	0.251
MMP1	**rs475007**	**A**	**T**	**chr11:102174522**	**42.57%**	**22**	**1.00**	**1.66**	**1.01**	**2.71**	**0.046**	**0.91**	**0.46**	**1.80**	**0.778**

Primary Analysis: Odds Ratio Estimates of Gene-NSAID Interaction

Marker Information							Case-Only Odds Ratios and P-Values (Major Allele as Reference)								
							AA	Genotype Aa				Genotype aa			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	Odds Ratio	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
MMP1	rs514921	A	G	chr11:102174440	27.47%	97	1.00	0.88	0.57	1.36	0.558	0.64	0.27	1.52	0.311
MMP1	**rs5854**	**C**	**T**	**chr11:102166084**	**37.64%**	**63**	**1.00**	**0.59**	**0.36**	**0.97**	**0.036**	**1.01**	**0.55**	**1.87**	**0.966**
MMP1	rs7125062	T	C	chr11:102168713	26.02%	70	1.00	1.48	0.93	2.38	0.102	1.84	0.80	4.25	0.152
MMP1	**rs7945189**	**C**	**T**	**chr11:102165774**	**11.53%**	**71**	**1.00**	**0.47**	**0.25**	**0.89**	**0.021**	**2.19**	**0.43**	**11.18**	**0.346**
MMP3	rs3025058	-	T	chr11:102221162	45.37%	51	1.00	0.83	0.50	1.36	0.456	1.03	0.54	1.96	0.924
MMP3	rs3025065	A	G	chr11:102216192	0.11%	17	1.00	-	-	-	-	-	-	-	-
MMP3	rs3025066	A	G	chr11:102215693	8.20%	87	1.00	1.29	0.72	2.31	0.398	-	-	-	-
MMP3	rs476762	T	A	chr11:102215917	11.55%	93	1.00	0.83	0.48	1.46	0.525	1.16	0.22	6.00	0.859
MMP3	rs522616	A	G	chr11:102220258	18.28%	25	1.00	0.84	0.51	1.38	0.482	1.37	0.51	3.65	0.533
MMP3	rs527832	C	T	chr11:102207094	9.24%	28	1.00	1.07	0.61	1.89	0.807	-	-	-	-
MMP3	rs591058	T	C	chr11:102216548	45.59%	30	1.00	0.86	0.52	1.43	0.557	1.05	0.56	1.98	0.877
MMP3	rs650108	G	A	chr11:102213997	24.49%	67	1.00	0.74	0.46	1.18	0.204	1.46	0.64	3.32	0.364
MMP9	rs13040272	T	C	chr20:44066518	42.23%	4	1.00	1.20	0.75	1.93	0.442	1.00	0.53	1.88	0.999
MMP9	rs13040572	A	C	chr20:44072376	36.26%	76	1.00	1.20	0.75	1.91	0.445	0.89	0.45	1.76	0.733
MMP9	rs13925	G	A	chr20:44078372	15.36%	77	1.00	1.35	0.82	2.22	0.233	0.87	0.24	3.20	0.839
MMP9	rs13969	C	A	chr20:44076240	0.00%	Failed									
MMP9	rs17576	A	G	chr20:44073632	36.37%	5	1.00	1.17	0.74	1.86	0.493	0.92	0.47	1.83	0.821
MMP9	rs1805088	C	T	chr20:44071031	2.80%	79	1.00	0.73	0.27	1.98	0.530	-	-	-	-
MMP9	rs1805089	G	A	chr20:44072017	0.00%	9	1.00	-	-	-	-	-	-	-	-
MMP9	rs2236416	A	G	chr20:44073982	15.80%	45	1.00	1.26	0.77	2.06	0.363	1.17	0.36	3.74	0.796
MMP9	rs2250889	C	G	chr20:44075813	5.31%	46	1.00	1.01	0.49	2.10	0.975	-	-	-	-
MMP9	rs2274756	G	A	chr20:44076518	15.69%	47	1.00	1.28	0.78	2.10	0.329	1.17	0.37	3.75	0.793
MMP9	rs3787268	G	A	chr20:44075138	20.32%	89	1.00	0.92	0.58	1.46	0.722	0.45	0.13	1.58	0.212
MMP9	rs3918242	T	C	chr20:44069383	0.00%	Failed									
MMP9	rs3918251	A	G	chr20:44072188	37.98%	55	1.00	1.45	0.91	2.32	0.116	1.11	0.57	2.16	0.767
MMP9	rs3918254	C	T	chr20:44073798	0.00%	90	1.00	-	-	-	-	-	-	-	-
MMP9	rs3918278	G	A	chr20:44069061	2.80%	91	1.00	0.95	0.37	2.42	0.906	-	-	-	-
MMP9	rs8125581	G	A	chr20:44072650	0.00%	32	1.00	-	-	-	-	-	-	-	-
MMP9	rs9509	T	C	chr20:44078560	3.06%	35	1.00	0.69	0.26	1.87	0.466	-	-	-	-
PLA2G7	rs1805018	T	C	chr6:46787262	4.17%	8	1.00	0.95	0.43	2.08	0.895	-	-	-	-
PON1	rs854542	A	G	chr7:94759427	21.96%	33	1.00	0.97	0.61	1.54	0.892	1.60	0.53	4.85	0.410
PTGIS	rs476496	A	G	chr20:47611191	27.58%	92	1.00	1.08	0.68	1.70	0.742	2.08	0.90	4.79	0.086
PTGIS	rs477627	C	T	chr20:47613465	18.96%	56	1.00	1.24	0.77	2.00	0.378	1.90	0.65	5.57	0.243
PTGIS	rs495146	C	T	chr20:47563735	20.40%	94	1.00	1.04	0.67	1.64	0.853	0.93	0.27	3.20	0.905
PTGIS	rs501908	A	G	chr20:47589174	7.93%	58	1.00	0.96	0.49	1.89	0.897	2.29	0.31	17.14	0.422
PTGIS	rs508757	A	G	chr20:47572378	19.08%	59	1.00	1.02	0.63	1.65	0.927	0.43	0.08	2.47	0.345
PTGIS	rs5602	T	C	chr20:47555385	46.33%	99	1.00	1.06	0.63	1.78	0.829	1.07	0.56	2.04	0.837

Primary Analysis: Odds Ratio Estimates of Gene-NSAID Interaction

Marker Information							Case-Only Odds Ratios and P-Values (Major Allele as Reference)								
							AA	Genotype Aa				Genotype aa			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	Odds Ratio	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
PTGIS	rs6019910	C	T	chr20:47623094	5.98%	64	1.00	1.02	0.52	2.01	0.954	-	-	-	-
PTGIS	rs6090996	G	A	chr20:47567189	18.62%	65	1.00	0.84	0.52	1.38	0.491	0.90	0.28	2.89	0.860
PTGIS	rs927068	G	T	chr20:47611381	24.16%	103	1.00	0.98	0.62	1.54	0.913	0.59	0.19	1.78	0.348
PTGS1	rs10306114	A	G	chr9:124172343	5.61%	72	1.00	0.76	0.37	1.58	0.467	-	-	-	-
PTGS1	**rs10306135**	**A**	**T**	**chr9:124177516**	**15.77%**	**73**	**1.00**	**0.88**	**0.52**	**1.47**	**0.619**	**6.91**	**1.35**	**35.38**	**0.020**
PTGS1	rs10306202	G	A	chr9:124199342	8.01%	36	1.00	1.06	0.59	1.90	0.841	-	-	-	-
PTGS1	rs1213266	G	A	chr9:124176705	8.62%	40	1.00	0.73	0.39	1.35	0.314	-	-	-	-
PTGS1	**rs12353214**	**C**	**T**	**chr9:124201691**	**8.00%**	**75**	**1.00**	**0.65**	**0.32**	**1.32**	**0.236**	**4.55**	**1.08**	**19.12**	**0.039**
PTGS1	rs1236913	C	T	chr9:124173300	6.89%	41	1.00	0.76	0.39	1.48	0.422	-	-	-	-
PTGS1	rs2282169	G	C	chr9:124180517	20.59%	48	1.00	0.99	0.62	1.57	0.959	2.71	0.88	8.35	0.082
PTGS1	rs3842787	C	T	chr9:124173328	5.85%	53	1.00	0.81	0.39	1.68	0.572	-	-	-	-
PTGS1	rs4836885	T	C	chr9:124186190	14.30%	23	1.00	1.41	0.87	2.31	0.168	2.48	0.54	11.30	0.241
PTGS1	rs5789	C	A	chr9:124183794	2.82%	62	1.00	2.21	0.92	5.28	0.075	-	-	-	-
PTGS1	rs6478565	A	G	chr9:124188453	17.80%	66	1.00	1.14	0.70	1.85	0.598	2.14	0.63	7.22	0.223
PTGS2	rs12042763	G	T	chr1:184918499	23.80%	2	1.00	0.86	0.54	1.37	0.525	1.34	0.56	3.23	0.508
PTGS2	rs20417	G	C	chr1:184916944	18.24%	80	1.00	1.52	0.96	2.40	0.072	0.87	0.18	4.15	0.863
PTGS2	rs20432	T	G	chr1:184912946	0.00%	Failed									
PTGS2	rs2066826	G	A	chr1:184912550	14.19%	Failed									
PTGS2	rs2206593	G	A	chr1:184909052	6.42%	14	1.00	1.01	0.52	1.97	0.977	-	-	-	-
PTGS2	rs2745557	G	A	chr1:184915844	15.80%	50	1.00	1.11	0.67	1.84	0.680	1.82	0.59	5.65	0.300
PTGS2	rs4648261	G	A	chr1:184915627	2.27%	19	1.00	0.49	0.14	1.71	0.261	-	-	-	-
PTGS2	**rs4648276**	**T**	**C**	**chr1:184912111**	**14.67%**	**20**	**1.00**	**1.66**	**1.02**	**2.69**	**0.041**	**-**	**-**	**-**	**-**
PTGS2	rs4648298	A	G	chr1:184908305	2.54%	Failed									
PTGS2	rs5273	T	C	chr1:184910391	0.11%	60	1.00	-	-	-	-	-	-	-	-
PTGS2	rs5275	T	C	chr1:184909681	36.07%	26	1.00	1.51	0.92	2.50	0.106	0.90	0.43	1.86	0.769
PTGS2	rs5277	G	C	chr1:184914820	15.43%	27	1.00	0.84	0.52	1.37	0.488	1.48	0.30	7.39	0.632
PTGS2	rs689466	A	G	chr1:184917374	19.75%	68	1.00	0.94	0.59	1.52	0.813	1.11	0.34	3.57	0.865
PTGS2	rs689470	C	T	chr1:184907681	3.03%	101	1.00	1.62	0.70	3.79	0.263	-	-	-	-
RETN	rs3219177	C	T	chr19:7640369	22.62%	52	1.00	1.41	0.90	2.21	0.139	0.61	0.20	1.84	0.380
Error (CYP2C9)*	rs10579140	C	-	chr11:65899405	0.00%	Failed									
Error (THBD)*	rs1330684	G	A	chr9:129619420	38.84%	42	1.00	1.21	0.75	1.96	0.434	0.76	0.37	1.57	0.461

*2 errors were made while submitting rs#'s: our intention was to genotype rs13306848 in THBD and rs1057910 in CYP2C9

Additional Analysis: Global genotype test and allele count test

Marker Information							Global Genotype Test	Allele Count Test†			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
AGT	rs699	T	C	chr1:228912417	43.33%	69	0.118	0.80	0.58	1.09	0.154
AGT	rs943580	A	G	chr1:228903667	42.33%	34	0.090	0.73	0.53	1.01	0.059
APOE	rs429358	T	C	chr19:50103781	1.98%	Failed	-	-	-	-	-
APOE	rs7412	C	T	chr19:50103919	10.00%	Failed	-	-	-	-	-
Chr9p21.3	**rs10757274**	**G**	**A**	**chr9:22086055**	**45.48%**	**1**	**0.019**	**0.65**	**0.47**	**0.89**	**0.007**
Chr9p21.3	**rs2383206**	**G**	**A**	**chr9:22105026**	**43.33%**	**49**	**0.029**	**0.68**	**0.50**	**0.93**	**0.016**
CRP	**rs1205**	**C**	**T**	**chr1:157948857**	**31.87%**	**3**	**0.007**	**1.60**	**1.17**	**2.18**	**0.003**
CYP2C9	rs1799853	C	T	chr10:96692037	1.03%	Failed	-	-	-	-	-
CYP2C9	rs1856908	G	T	chr10:96722721	36.46%	10	0.959	0.99	0.72	1.36	0.957
CYP2C9	rs1934963	T	C	chr10:96724666	21.38%	44	0.978	1.02	0.70	1.47	0.925
CYP2C9	rs1934967	C	T	chr10:96731416	20.95%	11	0.581	1.11	0.76	1.61	0.604
CYP2C9	rs1934968	G	A	chr10:96731807	10.27%	12	0.936	0.96	0.58	1.59	0.865
CYP2C9	rs2153628	A	G	chr10:96713414	19.06%	83	0.759	1.13	0.77	1.65	0.541
CYP2C9	rs2298037	C	T	chr10:96736068	18.24%	15	0.927	0.93	0.63	1.37	0.707
CYP2C9	rs2860968	T	C	chr10:96703571	14.30%	16	0.368	0.72	0.45	1.14	0.160
CYP2C9	rs4918766	G	A	chr10:96701874	39.28%	24	0.782	0.91	0.66	1.24	0.533
CYP2C9	rs9332197	T	C	chr10:96730898	5.72%	104	0.708	0.59	0.28	1.25	0.171
CYP2C9	rs9332238	G	A	chr10:96738482	20.80%	105	0.728	1.06	0.73	1.54	0.772
EDN1	rs5370	G	T	chr6:12404241	17.44%	61	0.252	0.73	0.47	1.13	0.154
EDN1	rs9369217	C	T	chr6:12391748	11.57%	106	0.442	0.88	0.54	1.43	0.602
EDN1	rs9380973	T	C	chr6:12392375	0.00%	Failed	-	-	-	-	-
ESR1	**rs11155814**	**A**	**G**	**chr6:152192877**	**12.24%**	**38**	**0.026**	**0.73**	**0.44**	**1.20**	**0.211**
ESR1	**rs3853248**	**T**	**C**	**chr6:152181579**	**12.24%**	**54**	**0.023**	**0.72**	**0.44**	**1.18**	**0.193**
ESR2	rs3020450	G	A	chr14:63838055	33.41%	86	0.835	1.00	0.73	1.38	0.979
ESR2	rs7154455	G	C	chr14:63806413	33.11%	102	0.884	1.01	0.73	1.39	0.968
IL18	rs360722	C	T	chr11:111531913	11.10%	88	0.857	0.90	0.56	1.47	0.684
IL18	rs543810	A	G	chr11:111514188	11.21%	98	0.866	0.95	0.59	1.53	0.824
KL	**rs211247**	**C**	**G**	**chr13:32480646**	**18.09%**	**82**	**0.046**	**1.59**	**1.08**	**2.32**	**0.018**
MMP1	rs10488	G	A	chr11:102173232	5.98%	37	0.205	1.67	0.94	2.97	0.083
MMP1	rs1051121	C	T	chr11:102171183	3.03%	74	0.087	0.36	0.11	1.20	0.096
MMP1	rs1144393	T	C	chr11:102174619	39.96%	39	0.670	0.98	0.72	1.33	0.885
MMP1	rs17293761	C	T	chr11:102164443	5.27%	78	0.764	0.72	0.34	1.51	0.385
MMP1	rs17879165	G	A	chr11:102166730	0.11%	6	0.989	-	-	-	-
MMP1	rs1799750	-	G	chr11:102175706	46.62%	7	0.813	1.01	0.75	1.38	0.927
MMP1	**rs2071230**	**A**	**G**	**chr11:102166169**	**6.70%**	**13**	**0.105**	**1.76**	**1.00**	**3.09**	**0.049**
MMP1	rs2071232	T	C	chr11:102170879	17.88%	81	0.332	1.26	0.85	1.88	0.256
MMP1	rs2408489	C	A	chr11:102162896	21.64%	84	0.321	1.37	0.94	1.98	0.099
MMP1	rs2408490	C	T	chr11:102177763	17.27%	85	0.762	0.88	0.58	1.33	0.536
MMP1	rs3213460	G	A	chr11:102174092	13.63%	18	0.980	0.97	0.62	1.52	0.895
MMP1	rs470221	G	A	chr11:102170480	17.00%	21	0.391	1.30	0.89	1.90	0.183
MMP1	rs475007	A	T	chr11:102174522	42.57%	22	0.051	1.04	0.76	1.41	0.815

Additional Analysis: Global genotype test and allele count test

Marker Information							Global Genotype Test	Allele Count Test†			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
MMP1	rs484915	A	T	chr11:102178458	44.44%	57	0.850	1.07	0.79	1.46	0.654
MMP1	rs498186	A	C	chr11:102174855	44.17%	95	0.952	1.00	0.73	1.35	0.979
MMP1	rs5031036	A	G	chr11:102171374	5.84%	96	0.257	1.57	0.87	2.84	0.131
MMP1	rs514921	A	G	chr11:102174440	27.47%	97	0.555	0.82	0.58	1.17	0.275
MMP1	rs5854	C	T	chr11:102166084	37.64%	63	0.077	0.90	0.66	1.24	0.522
MMP1	rs7125062	T	C	chr11:102168713	26.02%	70	0.153	1.40	0.99	1.97	0.057
MMP1	**rs7945189**	**C**	**T**	**chr11:102165774**	**11.53%**	**71**	**0.039**	**0.66**	**0.39**	**1.13**	**0.128**
MMP3	rs3025058	-	T	chr11:102221162	45.37%	51	0.667	0.99	0.72	1.35	0.937
MMP3	rs3025065	A	G	chr11:102216192	0.11%	17	0.989	-	-	-	-
MMP3	rs3025066	A	G	chr11:102215693	8.20%	87	0.699	1.12	0.65	1.93	0.687
MMP3	rs476762	T	A	chr11:102215917	11.55%	93	0.798	0.90	0.55	1.46	0.655
MMP3	rs522616	A	G	chr11:102220258	18.28%	25	0.597	0.98	0.66	1.47	0.933
MMP3	rs527832	C	T	chr11:102207094	9.24%	28	0.971	0.94	0.55	1.59	0.806
MMP3	rs591058	T	C	chr11:102216548	45.59%	30	0.744	1.00	0.74	1.37	0.979
MMP3	rs650108	G	A	chr11:102213997	24.49%	67	0.231	0.97	0.68	1.38	0.856
MMP9	rs13040272	T	C	chr20:44066518	42.23%	4	0.697	1.04	0.76	1.40	0.826
MMP9	rs13040572	A	C	chr20:44072376	36.26%	76	0.626	1.01	0.73	1.39	0.957
MMP9	rs13925	G	A	chr20:44078372	15.36%	77	0.474	1.18	0.79	1.78	0.415
MMP9	rs13969	C	A	chr20:44076240	0.00%	Failed	-	-	-	-	-
MMP9	rs17576	A	G	chr20:44073632	36.37%	5	0.711	1.02	0.75	1.39	0.904
MMP9	rs1805088	C	T	chr20:44071031	2.80%	79	0.530	0.73	0.27	1.97	0.537
MMP9	rs1805089	G	A	chr20:44072017	0.00%	9	-	-	-	-	-
MMP9	rs2236416	A	G	chr20:44073982	15.80%	45	0.648	1.20	0.80	1.79	0.380
MMP9	rs2250889	C	G	chr20:44075813	5.31%	46	0.999	0.95	0.47	1.91	0.884
MMP9	rs2274756	G	A	chr20:44076518	15.69%	47	0.609	1.21	0.81	1.81	0.351
MMP9	rs3787268	G	A	chr20:44075138	20.32%	89	0.447	0.80	0.54	1.19	0.275
MMP9	rs3918242	T	C	chr20:44069383	0.00%	Failed	-	-	-	-	-
MMP9	rs3918251	A	G	chr20:44072188	37.98%	55	0.278	1.15	0.84	1.56	0.390
MMP9	rs3918254	C	T	chr20:44073798	0.00%	90	-	-	-	-	-
MMP9	rs3918278	G	A	chr20:44069061	2.80%	91	0.906	0.95	0.37	2.40	0.908
MMP9	rs8125581	G	A	chr20:44072650	0.00%	32	-	-	-	-	-
MMP9	rs9509	T	C	chr20:44078560	3.06%	35	0.466	0.70	0.26	1.87	0.473
PLA2G7	rs1805018	T	C	chr6:46787262	4.17%	8	0.895	0.95	0.44	2.05	0.899
PON1	rs854542	A	G	chr7:94759427	21.96%	33	0.684	1.06	0.74	1.53	0.755
PTGIS	rs476496	A	G	chr20:47611191	27.58%	92	0.227	1.24	0.89	1.73	0.209
PTGIS	rs477627	C	T	chr20:47613465	18.96%	56	0.405	1.28	0.88	1.88	0.202
PTGIS	rs495146	C	T	chr20:47563735	20.40%	94	0.972	1.02	0.70	1.49	0.935
PTGIS	rs501908	A	G	chr20:47589174	7.93%	58	0.713	1.09	0.61	1.96	0.762
PTGIS	rs508757	A	G	chr20:47572378	19.08%	59	0.634	0.92	0.62	1.38	0.698
PTGIS	rs5602	T	C	chr20:47555385	46.33%	99	0.970	1.04	0.76	1.41	0.823
PTGIS	rs5628	C	T	chr20:47574089	5.07%	29	0.489	0.79	0.38	1.63	0.518

Additional Analysis: Global genotype test and allele count test

Marker Information							Global Genotype Test	Allele Count Test†			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
PTGIS	rs574113	A	G	chr20:47553590	34.81%	100	0.897	1.06	0.77	1.46	0.720
PTGIS	rs6019902	G	A	chr20:47611620	18.24%	31	0.998	1.00	0.67	1.49	1.000
PTGIS	rs6019910	C	T	chr20:47623094	5.98%	64	0.998	0.97	0.51	1.84	0.914
PTGIS	rs6090996	G	A	chr20:47567189	18.62%	65	0.785	0.88	0.59	1.32	0.534
PTGIS	rs927068	G	T	chr20:47611381	24.16%	103	0.643	0.89	0.62	1.27	0.502
PTGS1	rs10306114	A	G	chr9:124172343	5.61%	72	0.768	0.73	0.36	1.49	0.391
PTGS1	**rs10306135**	**A**	**T**	**chr9:124177516**	**15.77%**	**73**	**0.048**	**1.18**	**0.78**	**1.78**	**0.447**
PTGS1	rs10306202	G	A	chr9:124199342	8.01%	36	0.980	0.98	0.56	1.71	0.944
PTGS1	rs1213266	G	A	chr9:124176705	8.62%	40	0.314	0.76	0.43	1.35	0.347
PTGS1	rs12353214	C	T	chr9:124201691	8.00%	75	0.052	1.15	0.67	1.97	0.621
PTGS1	rs1236913	C	T	chr9:124173300	6.89%	41	0.422	0.78	0.41	1.47	0.446
PTGS1	rs2282169	G	C	chr9:124180517	20.59%	48	0.209	1.18	0.82	1.71	0.378
PTGS1	rs3842787	C	T	chr9:124173328	5.85%	53	0.852	0.74	0.36	1.50	0.400
PTGS1	rs4836885	T	C	chr9:124186190	14.30%	23	0.240	1.41	0.93	2.15	0.108
PTGS1	rs5789	C	A	chr9:124183794	2.82%	62	0.075	2.10	0.91	4.84	0.081
PTGS1	rs6478565	A	G	chr9:124188453	17.80%	66	0.450	1.23	0.83	1.81	0.312
PTGS2	rs12042763	G	T	chr1:184918499	23.80%	2	0.610	1.00	0.70	1.44	0.988
PTGS2	rs20417	G	C	chr1:184916944	18.24%	80	0.185	1.30	0.89	1.89	0.178
PTGS2	rs20432	T	G	chr1:184912946	0.00%	Failed	-	-	-	-	-
PTGS2	rs2066826	G	A	chr1:184912550	14.19%	Failed	-	-	-	-	-
PTGS2	rs2206593	G	A	chr1:184909052	6.42%	14	1.000	0.92	0.48	1.76	0.807
PTGS2	rs2745557	G	A	chr1:184915844	15.80%	50	0.565	1.21	0.80	1.83	0.361
PTGS2	rs4648261	G	A	chr1:184915627	2.27%	19	0.261	0.50	0.14	1.72	0.270
PTGS2	rs4648276	T	C	chr1:184912111	14.67%	20	0.124	1.27	0.83	1.94	0.265
PTGS2	rs4648298	A	G	chr1:184908305	2.54%	Failed	-	-	-	-	-
PTGS2	rs5273	T	C	chr1:184910391	0.11%	60	0.989	0.00	0.00	.	0.983
PTGS2	rs5275	T	C	chr1:184909681	36.07%	26	0.162	1.06	0.76	1.47	0.737
PTGS2	rs5277	G	C	chr1:184914820	15.43%	27	0.670	0.92	0.60	1.41	0.693
PTGS2	rs689466	A	G	chr1:184917374	19.75%	68	0.953	0.98	0.67	1.44	0.935
PTGS2	rs689470	C	T	chr1:184907681	3.03%	101	0.534	1.99	0.91	4.37	0.086
RETN	rs3219177	C	T	chr19:7640369	22.62%	52	0.187	1.09	0.76	1.56	0.645
Error (CYP2C9)*	rs10579140	C	-	chr11:65899405	0.00%	Failed	-	-	-	-	-
Error (THBD)*	rs1330684	G	A	chr9:129619420	38.84%	42	0.378	0.95	0.69	1.30	0.740

*2 errors were made while submitting rs#'s: our intention was to genotype rs13306848 in THBD and rs1057910 in CYP2C9

Note: Markers in bold were significant on either the global genotype test or the allele test or both.

†Reference allele is the major allele.

COX-2 Subgroup Analysis: Odds ratio estimates of gene-COX2 interaction

Marker Information							Case-Only Odds Ratios and P-Values (Major Allele as Reference)								
							AA	Genotype Aa				Genotype aa			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	Odds Ratio	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
AGT	rs943580	A	G	chr1:228903667	42.33%	34	1.00	1.35	0.80	2.29	0.260	0.73	0.34	1.60	0.438
AGT	rs699	T	C	chr1:228912417	43.33%	69	1.00	1.47	0.85	2.53	0.170	0.97	0.45	2.07	0.928
APOE	rs429358	T	C	chr19:50103781	1.98%	Failed									
APOE	rs7412	C	T	chr19:50103919	10.00%	Failed									
Chr9p21.3	rs10757274	G	A	chr9:22086055	45.48%	1	1.00	0.63	0.35	1.12	0.115	0.48	0.23	1.01	0.054
Chr9p21.3	rs2383206	G	A	chr9:22105026	43.33%	49	1.00	0.59	0.34	1.03	0.064	0.56	0.27	1.16	0.120
CRP	**rs1205**	**C**	**T**	**chr1:157948857**	**31.87%**	**3**	**1.00**	**1.49**	**0.88**	**2.50**	**0.135**	**3.59**	**1.64**	**7.86**	**0.001**
CYP2C9	rs1856908	G	T	chr10:96722721	36.46%	10	1.00	1.02	0.61	1.69	0.940	1.04	0.51	2.14	0.914
CYP2C9	rs1934967	C	T	chr10:96731416	20.95%	11	1.00	1.45	0.85	2.48	0.169	1.23	0.37	4.03	0.737
CYP2C9	rs1934968	G	A	chr10:96731807	10.27%	12	1.00	1.17	0.63	2.16	0.615	-	-	-	-
CYP2C9	rs2298037	C	T	chr10:96736068	18.24%	15	1.00	1.00	0.61	1.62	0.985	0.47	0.05	4.21	0.501
CYP2C9	rs2860968	T	C	chr10:96703571	14.30%	16	1.00	0.81	0.46	1.41	0.450	0.42	0.05	3.55	0.429
CYP2C9	rs4918766	G	A	chr10:96701874	39.28%	24	1.00	0.92	0.53	1.60	0.768	0.79	0.38	1.65	0.532
CYP2C9	rs1934963	T	C	chr10:96724666	21.38%	44	1.00	0.97	0.58	1.65	0.923	1.04	0.37	2.97	0.942
CYP2C9	rs2153628	A	G	chr10:96713414	19.06%	83	1.00	1.23	0.73	2.08	0.440	1.09	0.34	3.46	0.882
CYP2C9	rs9332197	T	C	chr10:96730898	5.72%	104	1.00	0.92	0.35	2.42	0.872	-	-	-	-
CYP2C9	rs9332238	G	A	chr10:96738482	20.80%	105	1.00	0.93	0.55	1.59	0.803	1.31	0.44	3.91	0.631
CYP2C9	rs1799853	C	T	chr10:96692037	1.03%	Failed									
EDN1	rs5370	G	T	chr6:12404241	17.44%	61	1.00	0.79	0.44	1.43	0.440	0.20	0.03	1.56	0.125
EDN1	rs9369217	C	T	chr6:12391748	11.57%	106	1.00	0.89	0.47	1.68	0.713	0.30	0.04	2.43	0.258
EDN1	rs9380973	T	C	chr6:12392375	0.00%	Failed									
ESR1	**rs11155814**	**A**	**G**	**chr6:152192877**	**12.24%**	**38**	**1.00**	**0.52**	**0.27**	**1.00**	**0.050**	**7.86**	**0.81**	**76.08**	**0.075**
ESR1	**rs3853248**	**T**	**C**	**chr6:152181579**	**12.24%**	**54**	**1.00**	**0.52**	**0.27**	**1.00**	**0.050**	**7.86**	**0.81**	**76.08**	**0.075**
ESR2	rs3020450	G	A	chr14:63838055	33.41%	86	1.00	1.31	0.80	2.16	0.280	1.08	0.47	2.49	0.862
ESR2	rs7154455	G	C	chr14:63806413	33.11%	102	1.00	1.34	0.82	2.20	0.244	0.99	0.41	2.37	0.981
IL18	rs360722	C	T	chr11:111531913	11.10%	88	1.00	0.96	0.52	1.79	0.896	-	-	-	-
IL18	rs543810	A	G	chr11:111514188	11.21%	98	1.00	1.03	0.56	1.90	0.917	-	-	-	-
KL	**rs211247**	**C**	**G**	**chr13:32480646**	**18.09%**	**82**	**1.00**	**2.31**	**1.37**	**3.90**	**0.002**	**3.09**	**0.78**	**12.20**	**0.108**
MMP1	rs17879165	G	A	chr11:102166730	0.11%	6	1.00	-	-	-	-	-	-	-	-
MMP1	rs1799750	-	G	chr11:102175706	46.62%	7	1.00	1.27	0.73	2.23	0.399	1.05	0.51	2.13	0.904
MMP1	rs2071230	A	G	chr11:102166169	6.70%	13	1.00	1.74	0.91	3.33	0.097	-	-	-	-
MMP1	rs3213460	G	A	chr11:102174092	13.63%	18	1.00	1.09	0.63	1.88	0.771	1.17	0.10	13.20	0.897
MMP1	rs470221	G	A	chr11:102170480	17.00%	21	1.00	1.21	0.74	2.00	0.449	2.23	0.58	8.52	0.242
MMP1	rs475007	A	T	chr11:102174522	42.57%	22	1.00	1.59	0.92	2.73	0.097	0.84	0.39	1.78	0.640
MMP1	rs10488	G	A	chr11:102173232	5.98%	37	1.00	1.14	0.55	2.39	0.724	6.00	0.54	66.14	0.144
MMP1	rs1144393	T	C	chr11:102174619	39.96%	39	1.00	1.15	0.66	1.97	0.627	0.71	0.33	1.53	0.382
MMP1	rs484915	A	T	chr11:102178458	44.44%	57	1.00	1.12	0.63	2.01	0.701	0.98	0.48	1.98	0.953
MMP1	rs5854	C	T	chr11:102166084	37.64%	63	1.00	0.58	0.34	1.01	0.053	0.79	0.39	1.61	0.519
MMP1	rs7125062	T	C	chr11:102168713	26.02%	70	1.00	1.63	0.97	2.75	0.066	1.69	0.68	4.23	0.262
MMP1	**rs7945189**	**C**	**T**	**chr11:102165774**	**11.53%**	**71**	**1.00**	**0.47**	**0.23**	**0.93**	**0.031**	**3.42**	**0.55**	**21.10**	**0.186**
MMP1	rs1051121	C	T	chr11:102171183	3.03%	74	1.00	0.49	0.14	1.74	0.268	-	-	-	-

COX-2 Subgroup Analysis: Odds ratio estimates of gene-COX2 interaction

Marker Information							Case-Only Odds Ratios and P-Values (Major Allele as Reference)								
							AA	Genotype Aa				Genotype aa			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	Odds Ratio	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
MMP1	rs17293761	C	T	chr11:102164443	5.27%	78	1.00	0.82	0.34	1.99	0.667	-	-	-	-
MMP1	rs2071232	T	C	chr11:102170879	17.88%	81	1.00	1.65	0.96	2.87	0.073	0.91	0.18	4.57	0.909
MMP1	rs2408489	C	A	chr11:102162896	21.64%	84	1.00	1.61	0.91	2.84	0.103	1.69	0.74	3.86	0.211
MMP1	rs2408490	C	T	chr11:102177763	17.27%	85	1.00	0.85	0.48	1.50	0.580	1.41	0.35	5.73	0.633
MMP1	rs498186	A	C	chr11:102174855	44.17%	95	1.00	1.13	0.64	1.98	0.680	1.04	0.52	2.10	0.914
MMP1	rs5031036	A	G	chr11:102171374	5.84%	96	1.00	1.03	0.48	2.20	0.948	6.00	0.54	66.14	0.144
MMP1	rs514921	A	G	chr11:102174440	27.47%	97	1.00	0.88	0.53	1.44	0.604	0.65	0.23	1.81	0.408
MMP3	rs3025065	A	G	chr11:102216192	0.11%	17	1.00	-	-	-	-	-	-	-	-
MMP3	rs522616	A	G	chr11:102220258	18.28%	25	1.00	0.80	0.46	1.39	0.424	1.95	0.63	6.02	0.243
MMP3	rs527832	C	T	chr11:102207094	9.24%	28	1.00	1.00	0.53	1.88	1.000	-	-	-	-
MMP3	rs591058	T	C	chr11:102216548	45.59%	30	1.00	0.86	0.49	1.52	0.603	1.21	0.59	2.49	0.606
MMP3	rs3025058	-	T	chr11:102221162	45.37%	51	1.00	0.89	0.51	1.56	0.683	1.25	0.60	2.62	0.554
MMP3	rs650108	G	A	chr11:102213997	24.49%	67	1.00	0.73	0.42	1.25	0.248	2.08	0.83	5.23	0.120
MMP3	rs3025066	A	G	chr11:102215693	8.20%	87	1.00	1.59	0.83	3.05	0.160	-	-	-	-
MMP3	rs476762	T	A	chr11:102215917	11.55%	93	1.00	0.83	0.44	1.57	0.563	0.96	0.10	9.24	0.969
MMP9	rs13040272	T	C	chr20:44066518	42.23%	4	1.00	1.02	0.61	1.73	0.931	0.80	0.39	1.63	0.536
MMP9	rs17576	A	G	chr20:44073632	36.37%	5	1.00	1.02	0.62	1.70	0.927	0.72	0.33	1.59	0.420
MMP9	rs1805089	G	A	chr20:44072017	0.00%	9	1.00	-	-	-	-	-	-	-	-
MMP9	rs8125581	G	A	chr20:44072650	0.00%	32	1.00	-	-	-	-	-	-	-	-
MMP9	rs9509	T	C	chr20:44078560	3.06%	35	1.00	0.98	0.35	2.74	0.965	-	-	-	-
MMP9	rs2236416	A	G	chr20:44073982	15.80%	45	1.00	1.29	0.74	2.25	0.376	0.63	0.14	2.95	0.560
MMP9	rs2250889	C	G	chr20:44075813	5.31%	46	1.00	0.87	0.37	2.04	0.752	-	-	-	-
MMP9	rs2274756	G	A	chr20:44076518	15.69%	47	1.00	1.29	0.74	2.25	0.376	0.63	0.14	2.95	0.560
MMP9	rs3918251	A	G	chr20:44072188	37.98%	55	1.00	1.33	0.79	2.24	0.277	0.83	0.38	1.78	0.626
MMP9	rs13040572	A	C	chr20:44072376	36.26%	76	1.00	1.00	0.59	1.68	0.994	0.74	0.34	1.64	0.460
MMP9	rs13925	G	A	chr20:44078372	15.36%	77	1.00	1.32	0.75	2.32	0.332	0.64	0.14	2.96	0.563
MMP9	rs1805088	C	T	chr20:44071031	2.80%	79	1.00	1.05	0.37	2.97	0.928				
MMP9	rs3787268	G	A	chr20:44075138	20.32%	89	1.00	0.81	0.48	1.37	0.430	0.38	0.08	1.70	0.203
MMP9	rs3918254	C	T	chr20:44073798	0.00%	90	1.00	-	-	-	-	-	-	-	-
MMP9	rs3918278	G	A	chr20:44069061	2.80%	91	1.00	0.93	0.33	2.62	0.897	-	-	-	-
MMP9	rs13969	C	A	chr20:44076240	0.00%	Failed									
MMP9	rs3918242	T	C	chr20:44069383	0.00%	Failed									
PLA2G7	rs1805018	T	C	chr6:46787262	4.17%	8	1.00	0.90	0.39	2.07	0.809	-	-	-	-
PON1	rs854542	A	G	chr7:94759427	21.96%	33	1.00	1.13	0.69	1.87	0.623	1.15	0.30	4.43	0.841
PTGIS	rs5628	C	T	chr20:47574089	5.07%	29	1.00	0.61	0.25	1.48	0.276	-	-	-	-
PTGIS	rs6019902	G	A	chr20:47611620	18.24%	31	1.00	0.77	0.43	1.36	0.361	0.26	0.03	2.05	0.202
PTGIS	rs477627	C	T	chr20:47613465	18.96%	56	1.00	1.24	0.73	2.10	0.437	1.76	0.47	6.62	0.403
PTGIS	rs501908	A	G	chr20:47589174	7.93%	58	1.00	0.80	0.37	1.72	0.563	2.22	0.30	16.61	0.437
PTGIS	rs508757	A	G	chr20:47572378	19.08%	59	1.00	0.85	0.49	1.48	0.568	0.62	0.09	4.27	0.627
PTGIS	rs6019910	C	T	chr20:47623094	5.98%	64	1.00	0.99	0.46	2.14	0.974	-	-	-	-
PTGIS	rs6090996	G	A	chr20:47567189	18.62%	65	1.00	0.82	0.47	1.44	0.492	0.79	0.21	2.99	0.725

COX-2 Subgroup Analysis: Odds ratio estimates of gene-COX2 interaction

Marker Information							Case-Only Odds Ratios and P-Values (Major Allele as Reference)								
							AA	Genotype Aa				Genotype aa			
Gene	Marker	Major Allele	Minor Allele	Position	MAF	SNP SAS CODE (S#)	Odds Ratio	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value	Odds Ratio	Lower Confidence Limit	Upper Confidence Limit	P-Value
PTGIS	rs476496	A	G	chr20:47611191	27.58%	92	1.00	1.21	0.72	2.01	0.475	1.63	0.60	4.43	0.337
PTGIS	rs495146	C	T	chr20:47563735	20.40%	94	1.00	0.87	0.52	1.46	0.600	0.62	0.13	2.93	0.544
PTGIS	rs5602	T	C	chr20:47555385	46.33%	99	1.00	0.86	0.49	1.50	0.590	0.72	0.35	1.50	0.382
PTGIS	rs574113	A	G	chr20:47553590	34.81%	100	1.00	0.99	0.56	1.75	0.974	1.74	0.83	3.62	0.141
PTGIS	rs927068	G	T	chr20:47611381	24.16%	103	1.00	0.82	0.49	1.38	0.457	0.28	0.06	1.26	0.098
PTGS1	rs4836885	T	C	chr9:124186190	14.30%	23	1.00	1.27	0.74	2.17	0.392	3.17	0.62	16.14	0.165
PTGS1	rs10306202	G	A	chr9:124199342	8.01%	36	1.00	1.06	0.56	2.00	0.869	-	-	-	-
PTGS1	rs1213266	G	A	chr9:124176705	8.62%	40	1.00	0.80	0.41	1.56	0.512	-	-	-	-
PTGS1	rs1236913	C	T	chr9:124173300	6.89%	41	1.00	0.86	0.41	1.80	0.692	-	-	-	-
PTGS1	**rs2282169**	**G**	**C**	**chr9:124180517**	**20.59%**	**48**	**1.00**	**0.96**	**0.57**	**1.61**	**0.873**	**3.88**	**1.09**	**13.82**	**0.037**
PTGS1	rs3842787	C	T	chr9:124173328	5.85%	53	1.00	1.14	0.53	2.43	0.740	0.00	0.00		0.993
PTGS1	rs5789	C	A	chr9:124183794	2.82%	62	1.00	1.71	0.68	4.34	0.257	-	-	-	-
PTGS1	rs6478565	A	G	chr9:124188453	17.80%	66	1.00	1.13	0.66	1.93	0.669	2.52	0.70	9.06	0.157
PTGS1	rs10306114	A	G	chr9:124172343	5.61%	72	1.00	1.03	0.48	2.18	0.949	-	-	-	-
PTGS1	**rs10306135**	**A**	**T**	**chr9:124177516**	**15.77%**	**73**	**1.00**	**0.88**	**0.49**	**1.58**	**0.678**	**6.94**	**1.35**	**35.65**	**0.020**
PTGS1	**rs12353214**	**C**	**T**	**chr9:124201691**	**8.00%**	**75**	**1.00**	**0.77**	**0.36**	**1.64**	**0.499**	**6.87**	**1.33**	**35.57**	**0.022**
PTGS2	rs12042763	G	T	chr1:184918499	23.80%	2	1.00	0.69	0.41	1.18	0.173	1.05	0.38	2.89	0.930
PTGS2	rs2206593	G	A	chr1:184909052	6.42%	14	1.00	0.93	0.44	1.97	0.851	-	-	-	-
PTGS2	rs4648261	G	A	chr1:184915627	2.27%	19	1.00	0.93	0.25	3.44	0.913	-	-	-	-
PTGS2	**rs4648276**	**T**	**C**	**chr1:184912111**	**14.67%**	**20**	**1.00**	**1.92**	**1.11**	**3.32**	**0.020**	**-**	**-**	**-**	**-**
PTGS2	rs5275	T	C	chr1:184909681	36.07%	26	1.00	1.65	0.93	2.92	0.086	1.10	0.49	2.47	0.812
PTGS2	rs5277	G	C	chr1:184914820	15.43%	27	1.00	0.72	0.41	1.25	0.238	1.43	0.16	12.55	0.749
PTGS2	rs2745557	G	A	chr1:184915844	15.80%	50	1.00	1.24	0.71	2.16	0.459	2.59	0.78	8.68	0.122
PTGS2	rs5273	T	C	chr1:184910391	0.11%	60	1.00	-	-	-	-	-	-	-	-
PTGS2	rs689466	A	G	chr1:184917374	19.75%	68	1.00	0.75	0.44	1.29	0.298	1.31	0.32	5.29	0.705
PTGS2	**rs20417**	**G**	**C**	**chr1:184916944**	**18.24%**	**80**	**1.00**	**1.70**	**1.02**	**2.85**	**0.044**	**1.50**	**0.29**	**7.87**	**0.634**
PTGS2	rs689470	C	T	chr1:184907681	3.03%	101	1.00	1.23	0.49	3.10	0.655	-	-	-	-
PTGS2	rs20432	T	G	chr1:184912946	0.00%	Failed									
PTGS2	rs2066826	G	A	chr1:184912550	14.19%	Failed									
PTGS2	rs4648298	A	G	chr1:184908305	2.54%	Failed									
RETN	rs3219177	C	T	chr19:7640369	22.62%	52	1.00	1.29	0.78	2.14	0.318	0.76	0.24	2.35	0.628
Error (CYP2C9)*	rs10579140	C	-	chr11:65899405	0.00%	Failed									
Error (THBD)*	rs1330684	G	A	chr9:129619420	38.84%	42	1.00	1.29	0.75	2.22	0.350	0.74	0.32	1.70	0.480

*2 errors were made while submitting rs#'s: our intention was to genotype rs13306848 in THBD and rs1057910 in CYP2C9

Appendix 4: Application to MUHC Pilot Project Competition

Clinical, Evaluative and Public Health Research

2006 Pilot Project Competition (PPC)

Project Title: Identification of Genetic Polymorphisms as a Predictor of Myocardial Infarction upon use of Selective Cox-2 Inhibitors

Main applicant: James Brophy MEng MD FRCP(c) FACC PhD
Associate Professor of Medicine
Divisions of Cardiology and Clinical Epidemiology
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Tel: (514) 842-1231 ext. 36771
Fax: (514) 843-1493

Competition stream: New Team of Investigators

New team investigators:

James C. Engert, PhD
McGill University

Peter Bogaty, MD
Laval University

Christine St.Germaine, MSc Candidate
McGill University

This group of investigators has not previously collaborated or published together.

Budget

1. Research staff	
Research assistant	$ 1,513.00
2. Materials, supplies, and services	
Services	$ 18,363.48
3. Equipment	
N/A	$ 0.00
Total costs	$ 19,876.48

Budget Justification:

1) Details of research staff:

Research assistant:

A research assistant (5% full-time equivalent) will be needed to coordinate activities involving specimen retrieval and organization. A salary of $1,513.00 is requested. This amount corresponds to 5% of the salary for a full-time research assistant (salary level #1 of $30,240 including 12% benefits).

2) Details of materials, supplies and services:

Services:

A total of $18,363.48 is requested for DNA extraction and the SNP genotyping and analysis at the Montreal Genome Quebec Innovation Center (using iPlex assay with the Sequenom MassArray technology).

DNA Extraction: $18.00 per sample * 368 samples	$ 6624.00
DNA Quantification and quality check:	$ 356.44
SNP Genotyping	$ 11,383.04
Total	**$ 18,363.48**

3) Details of equipment:

No financial assistance requested for equipment.

1. Background

Despite the efficacy of rofecoxib (Vioxx) for the treatment of osteoarthritis, menstrual pain, acute pain and rheumatoid arthritis, this cyclooxygenase (COX)-2 inhibitor was voluntarily withdrawn from the worldwide market due to the results of the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial which showed a significant increase in heart attacks and strokes among patients assigned to treatment with the drug versus patients assigned to placebo[1, 2]. This observation raised concerns about a class effect and prompted further investigations into the safety of all COX-2 selective non-steroidal anti-inflammatory drugs (NSAIDs).

Since their discovery, several generations of NSAIDs from aspirin and ibuprofen to selective COX-2 inhibitors, such as rofecoxib and celecoxib, have been widely used for fever, pain and inflammation. The biological mechanism responsible for the therapeutic efficacy of NSAIDs was first reported in the early 1970's when Vane et al demonstrated that NSAIDs reduce prostaglandin formation as a result of inhibition of the cyclooxygenase (COX) enzyme[3]. To date, two main isoforms of the COX enzyme, COX-1 and COX-2, have been identified. COX-1 is encoded by the constitutively expressed PTGS1 gene and produces prostaglandins (PGs) involved in the regulation of stomach mucosa, platelet aggregation, and kidney function[4]. COX-2 is encoded by the inducible PTGS2 gene and is rapidly induced by inflammatory cytokines and mitogens; it is, therefore, thought to be responsible for producing the majority of prostaglandins involved in inflammation and cancer[4]. Traditional NSAIDs target both COX isoforms, ranging from high selectivity towards COX-1 to equal activity on both. Because of the COX-1 enzyme's role in the regulation of stomach mucosa, it is believed that it is the inhibition of this isoform that is primarily responsible for the gastrointestinal side effects observed among NSAID users[5]. In 1999 a new class of NSAIDs, COX-2 selective inhibitors (coxibs), were introduced to the Canadian market. These "coxibs" have a higher affinity for

COX-2 than COX-1 and were designed to treat pain and inflammation without the GI side effects associated with traditional NSAIDs[3]. It is important to note that COX-2 binding affinity varies even among COX-2 selective agents and COX-2 selectivity is dose-dependent[6].

Although the severity of the cardiovascular events observed among COX-2 inhibitor users should not be ignored, the percentage of patients experiencing a thrombotic event in the APPROVe trial was fortunately low at 1.5% annually, suggesting that the majority of patients were treated without incident[2]. Furthermore, the biological mechanism by which these cardiovascular events are triggered is still unclear. Previous research has shown that individual therapeutic response to COX-2 inhibitors varies and this variation may be associated with genetic polymorphisms within the COX pathway[7]. Furthermore, a recent study found that rofecoxib significantly alters the expression of several genes related to the matrix metalloproteinase (MMP) pathway including MMP1, MMP3, PLAT, IL8, VIP, VIPR1, CD36 and TIMP3. Of these, MMP1 and MMP3 are known to be associated with inflammation and are likely to have roles in atherogenesis [8]. Therefore, it is plausible that the presence of one or more genetic polymorphisms may create an environment in which a COX-2 inhibitor will trigger cardiovascular events leading to a myocardial infarction or stroke.

Based on these results we hypothesize that there may be single nucleotide polymorphisms (SNPs) within one or more of the genes regulated by COX-2 inhibitors (MMP1, MMP3, PLAT, IL8, VIP, VIPR1, CD36, TIMP3) as well as the COX genes themselves (PTGS1, or PTGS2) which may cause the gene(s) to be expressed differently in response to coxibs and may induce the biological events necessary to provoke cardiovascular toxicity.

Although rofecoxib was withdrawn from the market in 2004, we have the unique opportunity to investigate a group of individuals from the RISCA (Récurrence et inflammation dans les syndromes coronariens aigus) prospective cohort collected between 2000 and 2002 who reported treatment with rofecoxib or celecoxib (Celebrex) at hospital admission for myocardial infarction and consented to blood collection for the purpose of genetic testing. With this cohort

we intend to conduct a pilot study on a subset of the RISCA cohort to identify candidate single nucleotide polymorphisms (SNPs) that may be predictive of cardiovascular events upon use of a COX-2 inhibitor. The findings of this study should advance our understanding of the genetic contribution to the cardiovascular risk of COX-2 inhibitors and will be useful in the development of a larger CIHR grant application.

2. Goals and Objectives

The goals of this pilot study are:

1) To identify candidate single nucleotide polymorphism(s) within the COX genes or genes regulated by COX-2 inhibitors (MMP1, MMP3, PLAT, IL8, VIP, VIPR1, CD36, TIMP3, PTGS1, and PTGS2) that may be predictive of MI upon use of rofecoxib or celecoxib.
2) To develop a new cardiovascular genetic epidemiology research team to investigate genetic predictors of adverse cardiovascular events among users of common pharmacological therapies.

In this pilot study, ten genes within or downstream of the COX pathway have been selected for SNP analysis which will be performed in a subset of patients from the RISCA cohort. If this pilot study is successful, additional funding will be sought to conduct high throughput testing in the entire RISCA cohort. Ultimately, we hope to identify genetic polymorphisms that are more frequent among individuals who incur a myocardial infarction while taking rofecoxib or celecoxib and, thus, may be candidates for genetic predictors of these adverse cardiovascular events. We hypothesize that if there are genetic polymorphisms that are more frequent among rofecoxib or celecoxib users who experience MI, the polymorphism(s) may indicate a genetic background in which the biological response to COX-2 inhibitors leads to cardiovascular complications. By identifying genetic markers that may predict increased susceptibility to COX-2 inhibitor-affiliated cardiovascular events, it may be possible to prospectively identify those patients who, according to their genotype, are at risk of a cardiovascular event and those whose risk remains unchanged.

3. Methods

A pilot study, nested within the prospective cohort of the RISCA study, will be performed. From 2000 to early 2002, the RISCA study recruited 1210 participants admitted to hospital for unstable angina (UA) or myocardial infarction (MI) from four tertiary and four community hospitals, seven of which are located in Quebec (Hopital Laval, Thetford Mines, Sagamie, Grand-Portage, Notre Dame, Drummondville, Montreal General) and one in New Brunswick. Attempts were made to recruit subjects consecutively in each center and a register of patients admitted but not recruited was maintained.

Subjects were recruited within 24 hours of symptom onset, according to a specified clinical definition for UA and MI. MI was defined as "characteristic discomfort or pain with an elevation of creatine kinase-MB (CK-MB) to 1.5 times the upper normal limit" and included both incident and recurrent cases. UA was defined as "either one episode lasting ≥40 min or ≥2 episodes lasting ≥5 min within 24h or characteristic discomfort or pain at rest with minimal exertion and at least one of these features: ECG changes; cardiac tropoinin I or T valued under the positive range for MI; history of MI or coronary revascularization; previous coronary angiogram with at least on ≥50% stenosis; previous non-invasive test showing myocardial ischemia or evidence of MI; known peripheral vascular disease; known non hemorrhagic cerebrovascular disease; presence of diabetes." Principal investigators of the RISCA study confirmed diagnoses and in the case of ambiguities an outside clinical researcher was used for adjudication. Of the 1210 recruits, 747 were admitted for MI and 416 for UA, with the remainder not meeting either clinical definition. Blood samples were collected from consenting subjects within 24 hours of symptom onset. A computerized blood storage system was used, ensuring precise and instantaneous inventory information and efficient retrieval while maintaining patient anonymity. Among those consenting, a total of 92 subjects (n=43 rofecoxib; n=49 celecoxib) reported treatment with a selective COX-2 inhibitor within 10 days prior to hospital admission for UA or MI.

In this **case-only** study, the "controls" will be subjects not exposed to coxibs at the time of hospital admission. We will randomly select 3 "controls" for each subject exposed to coxibs (3:1). Therefore, DNA samples will be retrieved for 368 subjects (n=276 not exposed to coxibs: n=92 exposed to coxibs) within the RISCA cohort and SNP analysis will be performed. SNPs selected for testing will be located in one of the following ten genes: MMP1, MMP3, PLAT, IL8, VIP, VIPR1, CD36, TIMP3, PTGS1, or PTGS2. An exploratory descriptive analysis will compare the frequencies of selected single nucleotide polymorphisms (SNPs), among individuals exposed to rofecoxib or celecoxib, with those who did not report treatment with a COX-2 inhibitor at baseline to determine if any of the SNPs from the ten hypothesized genes may be candidates for a predictor of adverse cardiovascular events upon coxib use. We will also use case-only analysis to estimate the strength of association between a particular genotype and exposure to rofecoxib or celecoxib.[9] This analysis assumes independence of genotype and the exposure and is used to assess departure from multiplicative effects[9]. It has been shown that a case-only design can estimate a gene-environment interaction more precisely than a cohort or case-control design[10].

The calculation of the sample size for case-only designs is a complex function of "the prevalence of exposure (e) and genotype (g), the relative risk for exposure alone (Re) and for genotype alone (Rg) and the effect of the gene-environment interaction (Ri), the type I error (alpha), and the type II error (beta)".[11] The power of our study is difficult to estimate at this time because it will depend on the prevalence of exposure to coxibs and the prevalence of each genotype we test. For example, for a genotype with a prevalence of 0.10, Yang et al report 80% power for 10% exposure rates with a sample size of 195 cases to detect a relative risk of 5 and 1568 cases to detect a RR of 2.[11] However, tests of genotypes with higher prevalence will have greater power. Nevertheless, our exploratory analysis will test approximately 10 SNPs per gene (depending on gene size) and, thus, multiple testing must be carefully considered. Therefore, we will focus on descriptive rather than statistical results.

4. Ethical Issues

Ethics approval was obtained for the RISCA trial. A separate form was used to obtain consent for the collection of DNA samples. Due to the addition of further genetic tests at the MUHC, the protocol will be submitted to the MUHC Royal Victoria Hospital Institutional Review Board (IRB) for ethics review.

It is not expected that results will pose any ethical problems, given the exploratory nature of this study. That is, if SNPs can be identified within the COX pathway that are associated with exposure to coxibs, this identification will merely direct future research. Any positive results would be primarily hypothesis generating and would not provide enough evidence to warrant informing patients or their physicians of a potential genetic susceptibility to MI with coxib use.

5. Conclusion

To the best of our knowledge, there are no published studies examining the gene-drug interaction between coxibs and genetic polymorphisms in patients who have had a myocardial infarction. Furthermore, due to the worldwide withdrawal, the opportunity to investigate genetic samples in rofecoxib users suffering a myocardial infarction is likely to be unique to the RISCA cohort. The successful completion of this pilot study will justify an application to CIHR for further investigation including high throughput genetic testing to identify any cardiovascular genes that may play a role in the adverse events observed among coxib users. Ultimately, an understanding of this potential gene environment interaction may provide some additional insights into the mechanisms whereby previously stable coronary atherosclerosis becomes unstable leading to clinical events.

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Appendix 5: Ethics

Centre universitaire de santé McGill
McGill University Health Centre
Les meilleurs soins pour la vie
The Best Care for Life

February 6, 2007

Dr. James Brophy
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Divisions of Cardiology and Clinical Epidemiology
CHUM / MUHC Technology Assessment Unit (Director)
Royal Victoria Hospital
687 Pine avenue West, Ross 4.12
Montreal (Qc)
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RE: GEN#06-047 entitled "Identification of Genetic Polymorphisms as a Predictor of Myocardial Infarction upon use of Non-steroidal Anti-inflammatory Drugs."

Dear Dr. Brohpy:

We are writing in response to your correspondence of November 20, 2006, requesting approval to use existing data and genetic samples extracted from subjects recruited between 2000 and 2002.

It is noted that all participants who were recruited to the RISCA study have agreed to provide a genetic sample and these genetic samples are presently being stored at Laval University. The samples will be sent to the MUHC where DNA extraction and analysis will be performed. No new subjects will be recruited, nor will the RISCA subjects be contacted during the study.

We are pleased to inform you that your request has been found ethically acceptable and we hereby grant you expedited approval to use the existing data and genetic samples extracted from subjects recruited between 2000 and 2002, via review by the Co-Chairman on February 6, 2007. We ask however that you provide a copy of the ethics approval(s) from the institution(s) where samples will be coming from.

Should any revision to the study, or other unanticipated development occur prior to the next required review, you must advise the REB without delay. Regulation does not permit initiation of a proposed study modification prior to REB approval for the amendment.

Good luck with your study.

Sincerely,

Denis Cournoyer, M. D.
Co-Chairman
GEN (Genetics/Population Research/Investigator Initiated Studies) Research Ethics Board
MUHC-Montreal General Hospital

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