Cardiac DNA Insight

Technical Bulletin

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ApoE, type III hyperlipoproteinemia and CVD risk

Report Type: Health Conditions

About: Cardiovascular disease refers to a set of conditions that affect the heart and blood vessels. It is the leading cause of death worldwide.[1] Risk factors for cardiovascular disease include smoking, being overweight, hypertension and high cholesterol and triglyceride levels.[2] Risk of cardiovascular disease is also significantly increased in patients with type III hyperlipoproteinemia (HLP), a condition diagnosed primarily by a biochemical test for the presence of triglyceride-rich particles in plasma called β-VLDL.[3] Type III HLP is also characterized by increased total cholesterol, LDL cholesterol, and triglyceride levels[4] and an elevated VLDL cholesterol to triglyceride ratio.[5] Through this elevation of blood lipids, type III HLP predisposes individuals to severe atherosclerosis and increased risk of cardiovascular disease[4]. This sustained cholesterol elevation also leads to development of xanthomas in some patients.[6] Genetic factors are significant contributors to the development of type III HLP-related cardiovascular disease.

Genetics: The apolipoprotein E (APOE) gene encodes a ligand for the LDL receptor that is needed for lipoprotein clearance. The gene has three common alleles, ε2, ε3 and ε4, that encode the E2, E3 and E4 isoforms of the protein, respectively. The APOE isoforms differ in their ability to bind lipoprotein receptors, with the E2 isoform binding with less than 2% of the strength of E3 or E4, which bind with approximately equal affinity.[7] Type III HLP is observed primarily in patients who are homozygous for ε2; more than 90% of affected patients are homozygous for ε2.[8] However, only 5% of patients with the homozygous genotype develop the disease.[4,7] It is thought, therefore, that at least one additional pathogenic genetic and/or environmental factor, such as diabetes, obesity, low estrogen level, or hypothyroidism, might be necessary for the disease to develop[4]. Indeed, clinical symptoms of type III HLP tend to present in men in the fourth decade of life and in women after menopause.[9,10]

Recommendations: For patients with an outcome of “Increased Risk”, consider earlier or increased frequency of screening for blood cholesterol levels, as suggested by the National Cholesterol Education Program (NCEP) Expert Panel and other groups.

Possible Outcomes: Increased Risk, Typical Risk

Markers Tested

Genotyping of APOE diplotype was performed in 86,067 healthy participants of mixed ethnicity (primarily from North America, Europe, and Asia),[11] as presented in the table below:

Gene[a]	Diplotype[b]	Population Frequency[c]	PMID[d]
APOE	e2/e2	0.007	17878422
APOE	e2/e3	0.116	17878422
APOE	e2/e4	0.022	17878422
APOE	e3/e3	0.623	17878422
APOE	e3/e4	0.213	17878422
APOE	e4/e4	0.019	17878422

[a]Gene containing the tested marker.

[b]Diplotype tested.

[c]Ethnicity of the population in the corresponding study.

[d]PubMed is a service managed by the National Library of Medicine; the PubMed ID (PMID) number identifies the referenced study.

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
429.2 Cardiovascular disease, unspecified • Arteriosclerotic cardiovascular disease [ASCVD] • Cardiovascular arteriosclerosis • Cardiovascular: ◦ degeneration (with mention of arteriosclerosis) ◦ disease (with mention of arteriosclerosis) ◦ sclerosis (with mention of arteriosclerosis)	N/A

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Atrial fibrillation

Report Type: Health Conditions

About: Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. This heart condition is the leading cause of arrhythmia-related hospitalizations and can contribute to stroke. Risk factors for AF include age, diabetes, hypertension and heart failure. Research indicates that genetic factors are also associated with AF.[1,2]

Genetics: The rs2200733 marker is associated with an increased risk of AF in many populations.[3,4] How rs2200733 affects heart rhythm is unknown and may be mediated through genes in the surrounding area. The closest known gene is PITX2, which is important in controlling asymmetry of the developing heart.[5] The next closest gene is ENPEP, which encodes an aminopeptidase responsible for the breakdown of angiotensin II in the vascular endothelium. However, mutations in these genes have not yet been shown to cause AF.

The T allele of rs2200733 was found to be associated with increased risk for atrial fibrillation in a large genome-wide association study conducted in Iceland.[3] These results were replicated in the same study in a second and larger Icelandic population, as well as in two more groups of individuals of European ancestry from Sweden and the US. Collectively, these results showed that a T allele at rs2200733 increases the risk of AF. The association appears stronger for earlier onset AF in both U.S. and Icelandic groups; individuals younger than 60 years had the highest relative risk. Additionally, each T allele carried a risk of diagnosis 2.28 years earlier. A large follow-up study from four cohorts of individuals of European descent was conducted, and in all four populations, rs2200733 was strongly associated with AF.[4] This study also showed an interaction with age of onset for the marker; younger individuals (less than 60 years old) had higher risk in three of the four populations studied. This marker was also studied in a Han Chinese population from Hong Kong. Although results were similar, the sample size for this population was too small to achieve statistical significance.[3]

Recommendations: NA

Possible Outcomes: Increased Risk, Above Average Risk, Average Risk

Markers Tested

Gene/ Locus[a]	Marker[b]	Associated Allele[c]	Odds Ratio[d]	Ethnicity[e]	Population Frequency[f]	Scientific Strength[g]	PMID[h]
PITX2	rs2200733	T	1.42	Asian	47.6%	Validated	17603472
PITX2	rs2200733	T	1.72	Caucasian	11.5%	Validated	17603472

[a]Gene or locus containing the tested marker.

[b]Marker tested.

[c]Allele associated with disease risk.

[d]Measure of the likelihood that an individual will get the disease if carrying a specific allele.

[e]Ethnicity of the population in the corresponding study.

[f]Percentage of people who have the associated allele in the population studied.

[g]Validated markers represent the highest quality genetic markers available; preliminary markers represent the latest in genetic research and have not met our highest standards for validation.

[h]PubMed is a service managed by the National Library of Medicine; the PubMed ID (PMID) number identifies the referenced study.

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
427.31 Atrial fibrillation	N/A

References

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Beta-blockers

(DC:TB-0233.002 07MAY2013)

Report Type: Pharmacogenetics

About: Beta-blockers (carvedilol, metoprolol, etc.) are used as a standard therapy for heart failure.[1] This class of drugs antagonizes beta-adrenergic receptors and down-regulates the effects of catecholamine hormones (epinephrine and norepinephrine). Genetic variants that affect beta-adrenergic signaling may influence outcomes in heart failure patients treated with beta-blockers.

Genetics: G-protein-coupled receptor kinases (GRKs) desensitize signaling of beta-adrenergic receptors. A gain-of-function variant in GRK5 (Gln41Leu) is associated with survival benefits in heart failure patients.[1,2] The Gln41Leu mutation is thought to provide a “natural” beta block, producing a more rapid desensitization of the beta-adrenergic receptor. In patients without the Gln41Leu mutation, beta-blockers provided patients with a typical survival benefit. In patients with the Gln41Leu mutation, beta-blockers were unlikely to provide additional survival benefit to heart failure patients. However, it is important to note that in heart failure patients who are not treated with beta-blockers, individuals with Gln41Leu had increased survival compared to those without.[1] The Gln41Leu mutation is more common in individuals of African ancestry, which may explain why African-Americans have a lower chance of responding to beta-blockers compared to other groups. This genetic effect is found in African-American patients;[1] it is not known if the tested mutation has the same effect in patients of non-African ancestry.

Recommendations: NA

Possible Outcomes: Reduced Therapeutic Benefit, Typical Therapeutic Benefit

Markers or Alleles Tested: GRK5 [rs17098707]

Ethnic Distribution of Tested Alleles: The minor allele (Leu41) frequency is 1.7% in Caucasians and 23.1% in African-Americans.[2]

Limitations and Warnings: The results of this test are based on a study of heart failure patients. Thus, they may not apply to patients being treated with beta-blockers for other conditions.

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
995.29 Unspecified adverse effect of other drug, medicinal and biological substance Applies to: • Unspecified adverse effect of medicinal substance NEC properly administered	N/A

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Caffeine metabolism

Report Type: Pharmacogenetics

About: Caffeine is the most widely consumed stimulant in the world. It acts as an adenosine receptor antagonist and thus modulates cAMP activity. The drug is metabolized to an inactive product by the liver enzyme cytochrome P450 1A2.[1]

Genetics: Cytochrome P450 1A2 is encoded by the CYP1A2 gene. Variants of CYP1A2 can affect enzyme activity and, thus, a patient's ability to metabolize caffeine. The A allele of the rs762551 marker in the CYP1A2 gene (CYP1A2*1F)† increases the activity of the enzyme, resulting in significantly enhanced caffeine metabolism.[1,2] Individuals homozygous for the A allele are classified as "fast" caffeine metabolizers, whereas individuals with a C allele (CYP1A2*1A) are classified as "slow" caffeine metabolizers.[3] However, increased caffeine metabolism by A allele homozygotes was observed in smokers but not in non-smokers, suggesting that in the absence of an inducer like smoking, A allele homozygotes may not be significantly different than C carriers.[2]

Coffee intake has been observed to be associated with increased risk for myocardial infarction in "slow" caffeine-metabolizing individuals with a C allele at rs762551 in the CYP1A2 gene.[3] In a study assessing the relationship between coffee drinking habits and myocardial infarction, researchers found that young (less than 59 years) individuals with the "slow" CYP1A2 genotype have an increased risk for heart attack if they are heavy coffee drinkers. Risk was greater for individuals who drank more than four cups of coffee per day than for individuals who drank two to three cups per day. The risk was not gender-specific and was not affected by smoking status.[3] Caffeine is the only major compound found in filtered coffee that is known to be metabolized by the cytochrome P450 1A2 (CYP1A2) enzyme, suggesting that caffeine is the component of coffee that increases the risk of heart attack.

†The CYP1A2 allele designation is based upon the recommendations of the International Human CYP Allele Nomenclature Committee[4] and is reflected in this report.

Recommendations: N/A

Possible outcomes: Slow Metabolizer, Fast Metabolizer

Markers or Alleles Tested: CYP1A2 [rs762551]

Ethnic Distribution of Tested Alleles: The C allele frequency at rs762551 was reported to be approximately 29% to 33% in Caucasians, 30% to 39% in Asians and 40% to 51% in Africans.[5]

Limitations and Warnings: An important consideration regarding A homozygotes ("fast" caffeine metabolizers) is that some studies have found that they do not differ from the C homozygotes ("slow" caffeine metabolizers) in the absence of a CYP1A2 inducer like tobacco or omeprazole.[2,6] Therefore, it is possible that "fast" caffeine metabolizers who are non-smokers, or not taking an inducer of CYP1A2, might have similar caffeine-associated, heart attack risk as the "slow" caffeine metabolizer.

Moreover, the A allele of rs762551 is also found in other haplotypes besides CYP1A2*1F (CYP1A2 Allele Nomenclature table).[7] Importantly, the frequency of the A allele of is not significantly different between Asians (63%, Koreans) and Caucasians (71%, Swedes), but the frequency of CYP1A2*1F haplotype was found to be significantly lower in Asians (0.4 to 7.7%) compared to Caucasians (57%).[5,8] In Caucasians, the A allele is mostly found in the CYP1A2*1F haplotype, while in Asians, it is mostly found in other haplotypes that contain additional mutations. In Asians, the CYP1A2*1F haplotype cannot be identified by testing for rs762551 alone and there is a lack of association of the A allele of rs762551 with increased enzyme activity or inducibility.[8] Therefore, this test is not recommended for Asians.

The CYP1A2 enzyme can metabolize a variety of compounds in addition to caffeine, such as the arthritis drug leflunomide. C homozygous individuals had a 9.7-fold higher risk for overall leflunomide-induced toxicity than did heterozygotes.[9] Some of the known CYP1A2 substrates, inhibitors and inducers are listed below in a table reproduced from Cytochrome P450 drug interaction table.[10]

In addition to genetics, a patient's ability to metabolize caffeine may also depend on nongenetic factors. Smoking, diet, oral contraceptives, and a variety of other drugs affect CYP1A2 activity.[5]

Known CYP1A2 Substrates	Known CYP1A2 Inhibitors	Known CYP1A2 Inducers
amitriptyline	fluvoxamine	broccoli
caffeine	ciprofloxacin	brussell sprouts
clomipramine	cimetidine	char-grilled meat
clozapine	amiodarone	insulin
cyclobenzaprine	fluoroquinolones	methylcholanthrene
estradiol	furafylline	modafinil
fluvoxamine	interferon	nafcillin
haloperidol	methoxsalen	beta-naphthoflavone
imipramine N-DeMe	mibefradil	omeprazole
mexilletine		tobacco
naproxen		
olanzapine		
ondansetron		
phenacetin		
acetaminophen→NAPQI		
propranolol		
riluzole		
ropivacaine		
tacrine		
theophylline		
tizanidine		
verapamil		
(R)warfarin		
zileuton		
zolmitriptan		

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Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
N/A	V77.99 Screening for other and unspecified endocrine, nutritional, metabolic, and immunity disorders

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Clopidogrel metabolism

(DC:TB-0237.001 06DEC2012)

Report Type: Pharmacogenetics

About: Clopidogrel (Plavix) is an oral anti-platelet agent used to inhibit blood clots in patients with coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Clopidogrel is a prodrug that must be metabolized to an active form to be effective. The CYP2C19 enzyme, a member of the cytochrome P450 superfamily, metabolizes clopidogrel to the active metabolite via a two-step reaction. Multiple cytochrome P450 enzymes contribute to the metabolism of clopidogrel, but CYP2C19 acts in both steps of the reaction, accounting for 45% of the first oxidation step and for 20% of the second step.[1]

Genetics: Variants of the CYP2C19 gene that lead to reduced enzyme function have been shown to be associated with reduced metabolism of clopidogrel to its active form in many ethnic populations.[2,3,4,5,6] Individuals can be classified based on their CYP2C19 enzyme activity into four metabolizer groups: Ultrarapid Metabolizer (higher than normal enzyme activity), Extensive Metabolizer (normal enzyme activity), Intermediate Metabolizer (intermediate enzyme activity) and Poor Metabolizer (low or no enzyme activity).[7]

In 2010, FDA added a boxed warning to the Plavix label indicating that clopidogrel can be less effective in CYP2C19 poor metabolizers and that genetic tests can help define a therapeutic strategy. In 2011, the NIH Clinical Pharmacogenetics Implementation Consortium issued guidelines describing clinical actions that can be implemented based on metabolizer status (see “Recommendations” below).[7]

The evidence is strongest for patients who are being treated with clopidogrel and receive percutaneous coronary intervention (PCI). Poor or intermediate metabolizers who receive PCI are at significantly increased risk of stent thrombosis, which can result in myocardial infarction and death.[8,9,10] For other indications, many studies have shown that poor metabolizers (2% to 15% of patients) and intermediate metabolizers (18% to 45% of patients) may be at risk for adverse cardiac events, such as myocardial infarction and stroke, when treated with clopidogrel;[7,11] however, recent studies dispute this claim.[9,12,13]

Recommendations: FDA’s boxed warning on the Plavix label recommends that alternative treatments should be considered for patients identified as CYP2C19 poor metabolizers.[14] The NIH Clinical Pharmacogenetics Implementation Consortium Guidelines recommend that prasugrel or another alternative be considered for intermediate and poor metabolizers.[7] The Royal Dutch Association for the Advancement of Pharmacy’s Pharmacogenetics Working Group also recommends alternative therapies, such as prasugrel.[15]

Concurrent use of clopidogrel with CYP2C19 inhibitors may affect clopidogrel response, particularly in extensive and ultra-rapid metabolizers. In these individuals, concurrent use of clopidogrel with CYP2C19 inhibitors (see "Known CYP2C19

Inhibitors" table below) may result in a poor metabolizer phenotype; as such, these individuals may have increased risk for cardiac adverse events when being treated with clopidogrel.[14]

Concurrent use of clopidogrel with omeprazole or esomeprazole should be avoided.[14] Concurrent use of clopidogrel with other CYP2C19 substrates (see "Known CYP2C19 Substrates" table below) may affect clopidogrel response.[16]

A patient's CYP2C19 metabolizer status may result in unexpected responses to other drugs, such as benzodiazepines, phenytoin, barbiturates and others[17] (see "Known CYP2C19 Substrates" table below).

Possible Outcomes: Poor Metabolizer, Intermediate Metabolizer, Extensive Metabolizer, Ultrarapid Metabolizer

Markers or Alleles Tested: CYP2C19 [CYP2C19*2, CYP2C19*3, CYP2C19*4, CYP2C19*5, CYP2C19*6, CYP2C19*8, CYP2C19*17]

Ethnic Distribution of Tested Alleles

The CYP2C19 panel detects alleles that have a combined frequency of over 99% in major ethnic groups.[7]

CYP2C19	Allele	Caucasian	African	East Asian	Middle Eastern	Enzyme activity
Wild-type	*1	63%	68%	60%	87%	Normal
rs4244285	*2	15%	15%	29%	12%	None
rs4986893	*3	0.42%	0.52%	8.9%	1.1%	None
rs28399504	*4	0.25%	0.093%	0.049%	ND[a]	None
rs56337013	*5	0.0073%	ND	0.062%	ND	Reduced
rs72552267	*6	0.017%	0%	0%	ND	None
rs41291556	*8	0.35%	0%	0%	ND	Reduced
rs12248560	*17	21%	16%	2.7%	ND	Increased

[a]Not Determined

Predicted CYP2C19 Metabolizer Status[7]

CYP2C19 Diplotype	Predicted Metabolizer Status
*2-*8/*2-*8	Poor Metabolizer
*1/*2-*8, *17/*2-*8	Intermediate Metabolizer
*1/*1	Extensive Metabolizer
*1/*17, *17/*17	Ultrarapid Metabolizer

Limitations and Warnings: Many rare CYP2C19 variants have been identified, but are not part of this test. It is possible, but unlikely, that the patient may have a variant that is not included in this test.

In addition to the genetic variants included in this test, other genetic and nongenetic factors can influence the effective dose of clopidogrel, including variants in other genes, age, sex, nutrition, lifestyle, other medications and route of administration.[16]

CYP2C19 genotype and metabolizer status may also affect responses to other drugs[17].

Known CYP2C19 Inhibitors and Substrates Tables

Known CYP2C19 Substrates[18]	Known CYP2C19 Inhibitors[18]
Proton-pump Inhibitors	Proton-pump Inhibitors
lansoprazole	lansoprazole
omeprazole	omeprazole
pantoprazole	pantoprazole
rabeprazole	rabeprazole
Anti-epileptics	Other
diazepam	chloramphenicol
phenytoin	cimetidine
S-mephenytoin	felbamate
phenobarbitone	fluoxetine
	fluvoxamine
Other	indomethacin
amitriptyline	ketoconazole
carisoprodol	modafinil
citalopram	oxcarbazepine
chloramphenicol	probenicid
clomipramine	ticlopidine
clopidogrel	topiramate
cyclophosphamide	
hexobarbital	
imipramine N-deME	
indomethacin	
R-mephobarbital	
moclobemide	
nelfinavir	
nilutamide	
primidone	
progesterone	
proguanil	
propranolol	
teniposide	
R-warfarin	

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Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
995.29 Unspecified adverse effect of other drug, medicinal and biological substance Applies to: • Unspecified adverse effect of medicinal substance NEC properly administered	N/A

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Coronary artery disease

Report Type: Health Conditions

About: Coronary artery disease (CAD), also known as coronary heart disease, is the leading cause of death in the U.S. This disease is a major consequence of atherosclerosis and can lead to chest pain and heart attack.[1] Risk factors for CAD are numerous, but family history is a major one. Research indicates that multiple genetic factors are associated with the CAD.[2]

Genetics: CAD is a complex genetic disorder. It is estimated that the genetic risk of atherosclerosis involves variants in hundreds of genes with a variety of functions in regulating blood pressure, lipid and cholesterol metabolism, pro-inflammatory processes and cell adhesion and migration.[2]

The test includes a variant at the 9p21 locus, a well-known genetic risk factor for CAD.[3] The association of 9p21 variants with early-onset CAD was first identified and replicated in a large genome-wide association study in the U.K. in 2007.[4] In this study, the C allele at the rs1333049 marker in the 9p21.3 region was associated with CAD in Caucasians. A later study of early-onset CAD replicated the risk for rs1333049 in Caucasians,[5] and yet another study that included patients from nine European study groups confirmed the association.[6] Genetic variants in the 9p21 region have also been associated with increased risk for many phenotypes related to CAD, including coronary atherosclerosis, CAD severity and progression, risk of intracranial or abdominal aortic aneurysms, as well as stroke.[3] Interestingly, the increased CAD risk conferred by 9p21 has been shown to be mitigated by a diet rich in fresh fruit and vegetables.[7]

It is not known what role 9p21 plays in cardiac function. The closest sites of interest to rs1333049 are a non-coding RNA of unknown function (ANRIL), which may regulate nearby genes, and CDKN2A and CDKN2B, which regulate the cell cycle.[8] Susceptibility at this site does not associate with other risk factors, so the effect is thought to be independent. The same region has also been associated with type 2 diabetes,[9] suggesting that there may be a shared mechanism involved in susceptibility for the diseases.

The U.K. study, among other studies, also identified additional markers included in the test. Of these, the MTHFD1L (methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like) gene (rs6922269) encodes a protein that affects serum levels of homocysteine, which is a known risk factor for cardiovascular disease.[4]

Recommendations: NA

Possible Outcomes: Increased Risk, Above Average Risk, Average Risk

Markers Tested

Gene/Locus[a]	Marker[b]	Associated Allele[c]	Odds Ratio[d]	Ethnicity[e]	Population Frequency[f]	Scientific Strength[g]	PMID[h]
Intergenic_9p21	rs1333049	C	1.30	Asian	51.2%	Validated	18264662
Intergenic_9p21	rs1333049	C	1.29	Caucasian	45.6%	Validated	18362232
HNF1A	rs2259816	A	1.08	Caucasian	38.1%	Validated	19198612
Intergenic_1q41	rs3008621	G	1.10	Caucasian	87.8%	Validated	19164808
Intergenic_10q11	rs501120	T[i]	1.11	Caucasian	83.3%	Validated	19164808
MTHFD1L	rs6922269	A	1.17	Caucasian	26.1%	Validated	17554300
MRAS	rs9818870	T	1.15	Caucasian	16.8%	Validated	19198612
SMAD3	rs17228212	C	1.19	Caucasian	33.9%	Preliminary	17634449
Intergenic_8p22	rs17411031	G	0.86	Caucasian	27.4%	Preliminary	17634449
Intergenic_2q36	rs2943634	C	1.22	Caucasian	65.5%	Preliminary	17634449
Intergenic_5q21	rs383830	A	1.60	Caucasian	79.2%	Preliminary	17554300
SEZ6L	rs688034	T	1.27	Caucasian	33.2%	Preliminary	17554300
CDH13	rs8055236	G	1.91	Caucasian	80.5%	Preliminary	17554300

[a]Gene or locus containing the tested marker

[b]Marker tested

[c]Allele associated with disease risk

[d]Measure of the likelihood that an individual will get the disease if carrying a specific allele

[e]Ethnicity of the population in the corresponding study

[f]Percentage of people who have the associated allele in the population studied

[g]Validated markers represent the highest quality genetic markers available; preliminary markers represent the latest in genetic research and have not met our highest standards for validation.

[h]PubMed is a service managed by the National Library of Medicine; thePubMed ID (PMID) number identifies the referenced study.

[i]This marker can be assayed on either strand of DNA. Therefore, the associated allele could be reported as either an A or a T in the patient report.

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
414.0 Coronary atherosclerosis Applies to: • Arteriosclerotic heart disease [ASHD] • Atherosclerotic heart disease • Coronary (artery): ◦ arteriosclerosis ◦ arteritis or endarteritis ◦ atheroma ◦ sclerosis ◦ stricture	N/A

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Genetic risk for decreased HDL cholesterol

Report Type: Metabolic Health Factors

About: High levels of high-density lipoprotein (HDL) cholesterol may protect against heart attack, while low levels may increase the risk of heart disease.[1] Though multiple mechanisms are known to account for the effects of HDL cholesterol levels, the major one is thought to be the role of HDL in transporting excess cholesterol away from the arteries and back to the liver, where it is passed from the body.[2] According to the National Cholesterol Education Program (NCEP) guidelines, levels lower than 40 mg/dl (for men) and lower than 50 mg/dl (for women) are considered risk factors for heart disease.[3]

Genetics: Fourteen genetic variants associated with decreased HDL cholesterol levels were identified in a large genome-wide association study that included over 19,000 Caucasian individuals from the Framingham Heart Study.[4] The association was replicated in another set of over 20,000 Caucasian individuals within the same study. While the function of these genetic variants is still being investigated, most are in genes such as CETP (cholesteryl ester transfer protein), FADS1 (fatty acid desaturase 1), LIPC (hepatic lipase), LIPG (endothelial lipase), LPL (lipoprotein lipase), PLTP (phospholipid transfer protein), amongst others, that are known to be involved in lipid metabolism.

An allele counting algorithm, in which risk alleles are weighted based on their effect size, was used to assign risk outcomes for each patient. This algorithm is based on a genome-wide association study (GWAS) that identified a set of 14 loci associated with HDL cholesterol levels. The authors used the alleles at each locus to assign a cumulative allelic dosage score for each individual. Based on dosage scores, individuals were divided into deciles and assessed for HDL cholesterol concentrations. The authors observed a significant trend in average HDL cholesterol concentration relative to allelic dosage score. Additionally, individuals with higher allelic dosage scores were more likely to have HDL levels below 40 mg/dl, a risk factor for heart disease.[4]

An outcome of "High Risk" indicates that the patient has a genetic profile similar to individuals in the study who fell into the two highest allelic dosage deciles. The average HDL cholesterol levels of these individuals were below 46 mg/dl. Approximately 37% of individuals in this group had levels below 40 mg/dl.[4] An outcome of "Above Average Risk" indicates that the patient has a genetic profile similar to individuals in the study who fell into the two next highest deciles; these individuals had HDL cholesterol levels that were, on average, below 50 mg/dl. Additionally, approximately 30% of individuals in this group had HDL cholesterol levels below 40 mg/dl.[4] An outcome of “Average Risk”, “Below Average Risk” or “Low Risk” indicates that the patient has a genetic profile similar to individuals in the study whose HDL cholesterol levels were, on average, above 50 mg/dl.[4]

Possible Outcomes: High Risk, Above Average Risk, Average Risk, Below Average Risk, Low Risk

Recommendations: Routine screening for blood cholesterol levels should be performed at appropriate ages, as recommended by the U.S. Preventive Services Task Force and other groups.[5]

Markers Tested and Scientific Strength

Gene/Locus[a]	Marker[b]	Risk Allele[c]	Scientific Strength[d]
ABCA1	rs1883025	A	4
ANGPTL4	rs2967605	A	4
CETP	rs173539[e]	C	4
FADS1	rs174547	C	4
GALNT2	rs4846914	G	4
HNF4A	rs1800961	T	4
KCTD10	rs2338104	C	4
LCAT	rs2271293	G	4
LIPC	rs10468017	C	4
LIPG	rs4939883	T	4
LPL	rs12678919	A	4
PLTP	rs7679	C	4
TTC39B	rs471364	G	4
ZNF259	rs964184	G	4

[a]Gene or locus containing the tested marker

[b]Marker tested

[c]"Risk Allele" refers to the allele that is associated with increased risk for the condition.

[d]"Scientific Strength" refers to the strength of research evidence for the genetic marker and the associated result. A rating of "4" indicates a study of over 2,000 people and at least one study that replicated the results. A rating of "3" indicates a study of over 400 people. A rating of "2" indicates a study of less than 400 people; studies in this category are preliminary but pass the criteria for statistical significance. A rating of "1" indicates that results are extremely preliminary.

[e]A proxy marker (rs247616) may be used on the test. The rs247616 marker can be assayed on either strand of DNA. Therefore, the associated allele for rs247616 could be reported as either a C or a G in the patient report.

Limitations and Warnings: These genetic variants together account for approximately 9.3% of the variance in HDL cholesterol levels[4] and, therefore, need to be considered together with other known risk factors for decreased HDL cholesterol levels. Specifically, an outcome of "High Risk" or "Above Average Risk" does not indicate that the patient has decreased HDL cholesterol levels; rather it indicates that the patient may have a genetic propensity for decreased HDL cholesterol levels. Similarly, an outcome of "Low" or "Below Average" does not indicate that the patient has optimal HDL cholesterol levels; rather it indicates that the patient has a lower than average genetic likelihood for decreased HDL cholesterol levels. To identify a patient's actual blood HDL cholesterol levels, a standard blood test could be considered.

The genetic risk for decreased HDL cholesterol has been studied and observed in Caucasian populations. The interpretation and recommendations are made in the context of Caucasian studies, and the results may or may not be relevant to tested individuals who are of non-Caucasian or mixed ethnicities.

References

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Genetic risk for elevated LDL cholesterol

Report Type: Metabolic Health Factors

About: At high levels, low-density lipoprotein (LDL) cholesterol can put a patient at risk for conditions such as heart attack or stroke. According to the National Cholesterol Education Program (NCEP) guidelines,[1] optimal LDL levels should be less than 100 mg/dl. Near-optimal levels range from 100 to 129 mg/dl and borderline-high from 130 to 159 mg/dl. A score greater than 160 mg/dl is high, and a score greater than 190 mg/dl is considered very high.

Genetics: Ten genetic variants associated with elevated LDL cholesterol levels were identified in a large genome-wide association study that included over 19,000 Caucasian individuals from the Framingham Heart Study.[2] The association was replicated in another set of over 20,000 Caucasian individuals within the same study. While the function of these genetic variants is still being investigated, most are in genes that are directly involved in lipid metabolism, such as APOB (apolipoprotein B) and LDLR (low density lipoprotein receptor). Others, such as HNF1A (hepatic nuclear transcription factor 1A), regulate genes involved in lipid metabolism.

An allele counting algorithm, in which risk alleles are weighted based on their effect size, was used to assign risk outcomes for each patient. An outcome of “High Risk” indicates that the patient has a genetic profile similar to individuals in the study whose LDL cholesterol levels were, on average, borderline-high. Approximately 25% of individuals in this group had levels in the high range.[2] An outcome of "Above Average Risk" indicates that the patient has a genetic profile similar to individuals in the study whose LDL cholesterol levels were on average borderline-high; approximately 17% of individuals in this group had levels in the high range.[2] An outcome of "Average Risk", “Below Average Risk” or “Low Risk” indicates that the patient has a genetic profile similar to individuals in the study whose LDL cholesterol levels were on average in the near-optimal range.[2]

Recommendations: Routine screening for blood cholesterol levels should be performed at appropriate ages, as recommended by the U.S. Preventive Services Task Force and other groups.[3]

Possible Outcomes: High Risk, Above Average Risk, Average Risk, Below Average Risk, Low Risk

Markers Tested and Scientific Strength

Gene/Locus[a]	Marker[b]	Risk Allele[c]	Scientific Strength[d]
ABCG8	rs6544713	T	4
APOB	rs515135	G	4
CELSR2	rs12740374	G	4
HMGCR	rs3846663	T	4
HNF1A	rs2650000	A	4
intergenic	rs1501908	G	4
LDLR	rs6511720	G	4
MAFB	rs6102059	C	4
NCAN	rs10401969	T	4
PCSK9	rs11206510	T	4

[a]Gene or locus containing the tested marker

[b]Marker tested

[c]"Risk Allele" refers to the allele that is associated with increased risk for the condition.

[d]"Scientific Strength" refers to the strength of research evidence for the genetic marker and the associated result. A rating of "4" indicates a study of over 2,000 people and at least one study that replicated the results. A rating of "3" indicates a study of over 400 people. A rating of "2" indicates a study of less than 400 people; studies in this category are preliminary but pass the criteria for statistical significance. A rating of "1" indicates that results are extremely preliminary.

Limitations and Warnings: These genetic variants together account for approximately 7.7% of the variance in LDL cholesterol levels[2] and, therefore, need to be considered together with other known risk factors for elevated LDL cholesterol levels. Specifically, an outcome of "High Risk" or "Above Average Risk" does not indicate that the patient has elevated LDL cholesterol levels; rather it indicates that the patient may have a genetic propensity for elevated LDL cholesterol levels. Similarly, an outcome of "Low Risk" or "Below Average Risk" does not indicate that the patient has optimal LDL cholesterol levels; rather it indicates that the patient has a lower than average genetic likelihood for elevated LDL cholesterol levels. To assess a patient's actual LDL cholesterol levels, a standard blood test could be considered.

The genetic risk for elevated LDL cholesterol has been studied and observed in Caucasian populations. The interpretation and recommendations are made in the context of Caucasian studies, and the results may or may not be relevant to tested individuals who are of non-Caucasian or mixed ethnicities.

References

1. ATP III at-a-glance: quick desk reference. National Heart Lung and Blood Institute web site. http://www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm. Updated 2004. Accessed August 3, 2012.
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Genetic risk for elevated triglycerides

Report Type: Metabolic Health Factors

About: Elevated triglycerides are a risk factor for conditions such as coronary artery disease and type 2 diabetes. According to the National Cholesterol Education Program (NCEP) guidelines,[1] a normal triglyceride score is under 150 mg/dl. Triglyceride levels in the range of 150 to 199 mg/dl are defined as borderline-high, with over 200 mg/dl considered high and over 500 mg/dl very high.

Genetics: Eleven genetic variants associated with elevated triglyceride levels were identified in a large genome-wide association study that included over 19,000 Caucasian individuals from the Framingham Heart Study.[2] The association was replicated in another set of over 20,000 Caucasian individuals within the same study. While the function of these genetic variants is still being investigated, most are in genes such as APOB (apolipoprotein B), FADS1 (fatty acid desaturase 1), LPL (lipoprotein lipase), PLTP (phospholipid transfer protein), amongst others, that are known to be involved in lipid metabolism.

An allele counting algorithm, in which risk alleles are weighted based on their effect size, was used to assign risk outcomes for each patient. An outcome of "High Risk" indicates that the patient has a genetic profile similar to individuals in the study whose triglyceride levels were, on average, borderline-high. Approximately 31% of individuals in this group had levels in the high range.[2] An outcome of “Above Average Risk”, “Average Risk”, “Below Average Risk” or “Low Risk” indicates that the patient has a genetic profile similar to individuals in the study whose triglyceride levels were on average under 150 mg/dl.[2]

Possible Outcomes: High Risk, Above Average Risk, Average Risk, Below Average Risk, Low Risk

Recommendations: N/A

Markers Tested and Scientific Strength

Gene/Locus[a]	Marker[b]	Risk Allele[c]	Scientific Strength[d]
ANGPTL3	rs10889353	A	4
APOB	rs7557067	A	4
FADS1	rs174547	C	4
GCKR	rs1260326	T	4
LPL	rs12678919	A	4
MLXIPL	rs714052	T	4
NCAN	rs17216525	C	4
PLTP	rs7679	C	4
TRIB1	rs2954029	A	4
XKR6	rs7819412	A	4
ZNF259	rs964184	G	4

[a]Gene or locus containing the tested marker

[b]Marker tested

[c]"Risk Allele" refers to the allele that is associated with increased risk for the condition.

[d]"Scientific Strength" refers to the strength of research evidence for the genetic marker and the associated result. A rating of "4" indicates a study of over 2,000 people and at least one study that replicated the results. A rating of "3" indicates a study of over 400 people. A rating of "2" indicates a study of less than 400 people; studies in this category are preliminary but pass the criteria for statistical significance. A rating of "1" indicates that results are extremely preliminary.

Limitations and Warnings: These genetic variants together account for approximately 7.4% of the variance in triglyceride levels[2] and, therefore, need to be considered together with other known risk factors for elevated triglyceride levels. Specifically, an outcome of "High Risk" or "Above Average Risk" does not indicate that the patient has elevated triglyceride levels; rather it indicates that the patient may have a genetic propensity for elevated triglyceride levels. Similarly, an outcome of "Low Risk" or "Below Average Risk" does not indicate that the patient has optimal triglyceride levels; it indicates that the patient has a lower than average genetic likelihood for elevated triglyceride levels. To assess a patient's actual triglyceride levels, a standard blood cholesterol test could be considered.

The genetic risk for elevated triglyceride levels has been studied and observed in Caucasian populations. The interpretation and recommendations are made in the context of Caucasian studies, and the results may or may not be relevant to tested individuals who are of non-Caucasian or mixed ethnicities.

References

1. ATP III at-a-glance: quick desk reference. National Heart Lung and Blood Institute web site. http://www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm. Updated 2004. Accessed August 3, 2012.
2. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nature genetics.* 2009;41:56-65.

Estrogen supplementation

(DC:TB-0228.001 06DEC2012)

Report Type: Pharmacogenetics

About: Combined hormonal contraceptives and post-menopausal hormone replacement therapy products contain estrogen. These medications by themselves pose an increased risk of blood clots (venous thrombosis), but the risk is even greater when they are used by individuals with certain inherited blood clotting disorders. Relatively common variants in two genes that code for components of the blood clotting cascade confer this increased risk.[1]

Genetics: The Factor V Leiden variant in the F5 gene, which encodes the factor V coagulation cofactor, and the G20210A mutation in the F2, or prothrombin gene, are associated with an increased risk of venous thrombosis in women using combined oral contraceptives or hormone replacement therapy.[2,3,4] Both the Factor V Leiden and the G20210A variants have dominant inheritance patterns, meaning that inheriting only one copy of the variant places a woman at increased risk of experiencing venous thrombosis if she uses estrogen supplementation.

By themselves, combined oral contraceptives increase the risk for blood clots in women 4-fold. Women who carry the Factor V Leiden mutation alone have an 8-fold increase in the risk for blood clots. Women who carry the Factor V Leiden mutation and who use combined oral contraceptives increase their risk for blood clots 35-fold.[1,5] In a meta-analysis of six case-control studies and one cohort study, presence of Factor V Leiden alone increased the risk of venous thromboembolism in combined oral contraceptive users over non-users.[1] In three case-control studies and one cohort study, the F2 G20210A variant alone also increased the risk of venous thromboembolism in combined oral contraceptive users over non-users.[6]

Post-menopausal hormone replacement therapy with oral estrogen also presents a risk to women with the Factor V Leiden and G20210A mutations. In women undergoing estrogen hormone replacement therapy, the Factor V Leiden mutation is associated with increased risk for deep vein thrombosis.[1,7] Additionally, a meta-analysis of six independent studies found that the Factor V Leiden and G20210A mutations increased the risk of blood clots in women undergoing estrogen hormone replacement therapy.[4] In an observational study of postmenopausal women carrying the Factor V Leiden or G20210A mutations, oral estrogen but not transdermal estrogen conferred additional risk of venous thromboembolism.[8]

Recommendations: According to the World Health Organization (WHO),[9] the U.K. Medical Eligibility Criteria[10] and the U.S. Centers for Disease Control and Prevention (CDC),[11] the use of combined oral contraceptives, the combined contraceptive patch or the combined contraceptive vaginal ring in individuals with known thrombogenic mutations (e.g., Factor V Leiden, prothombin mutation, protein S, protein C and antithrombin deficiencies) is an unacceptable health risk (level 4). The WHO also concludes that the evidence for combined oral contraceptives applies to combined injectable contraceptives. The Royal Dutch Association for the Advancement of Pharmacy's Pharmacogenetics Working Group recommends selecting alternative therapies to estrogen-containing oral contraceptives when a patient has the Factor V Leiden mutation and a family history of thrombotic events.[12]

The North American Menopause Society[13] and the Endocrine Society[14] acknowledge that thrombogenic mutations, such as Factor V Leiden, can increase the risk of venous thromboembolism, but they make no recommendations against using hormone replacement therapy in women with thrombogenic mutations. They do recommend thrombophilia screening prior to hormone replacement therapy use for women with a personal or family history of venous thromboembolism. For women with thromboembolic risk factors, the International Menopause Society suggests non-oral routes of estrogen or tibolone may be used if hormone replacement therapy is considered appropriate.[15]

The prevalence of individuals with one copy of both the Factor V Leiden and G20210A mutations is 1 in 1,000.[11]

Possible Outcomes: Increased Risk of Venous Thrombosis, Normal Risk of Venous Thrombosis

Markers and Alleles Tested: F5 [Factor V Leiden/R506Q]; F2 [G20210A]

Ethnic Distribution of Tested Alleles: The carrier rate for Factor V Leiden is 1 in 19 U.S. Caucasians, 1 in 45 Hispanic Americans, 1 in 83 African Americans, 1 in 222 Asian Americans and 1 in 80 Native Americans. In Europe, the mutation is particularly common with a carrier rate of 1 in 6 to 1 in 10 in southern Sweden and Greece and 1 in 33 to 1 in 50 in Italy and Spain. Similar high numbers have been found in many Middle Eastern countries.[16]

The carrier rate for G20210A is 2% to 5% in U. S. Caucasians. The mutation is found in 2% to 4% of healthy individuals in southern Europe, which is twice as high as the prevalence in northern Europe. G20210A is rare in Far Eastern populations, in Africa, and in indigenous populations of Australia and the Americas.[16]

Limitations and Warnings: Genetic variants in other proteins, such as protein S, protein C and antithrombin are known to increase the risk of venous thrombosis, but are not part of this test. Non-genetic factors known to increase the risk of venous thrombosis include age, obesity, trauma/surgery, smoking, pregnancy and airplane travel.[4]

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
E932.2 Ovarian hormones and synthetic substitutes causing adverse effects in therapeutic use Applies to: • Contraceptives, oral • Estrogens • Estrogens and progestogens combined • Progestogens	V78.9 Screening for unspecified disorder of blood and blood-forming organs

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Genetic risk for decreased folate

Report Type: Nutrition

About: Folate, a B-vitamin, plays a role in protein metabolism and DNA repair[1] and can lower the blood level of homocysteine, a substance linked to cardiovascular disease at high levels.[2] Diets rich in folate have also been associated with reduced risk of cardiovascular disease.[3] The vitamin is particularly important early in pregnancy for preventing some birth defects[1]. The recommended dietary allowance for most adults is 400 micrograms per day, while 600 micrograms of folate per day is recommended by the Institute of Medicine for pregnant women.

Genetics: The C677T variant in the methylenetetrahydrofolate reductase gene (MTHFR, which encodes a folate-metabolizing enzyme), has been associated with lowered folate levels in the blood in a study that included over six thousand Caucasian, African and Hispanic individuals from the third National Health and Nutrition Examination Survey (NHANES III).[2] The study also showed that dietary intake of folic acid could significantly reduce the negative impact of this variant on serum folate levels in individuals taking supplements containing greater than 400 micrograms folate per day. Therefore, people with a T allele are recommended to optimize their intake of folate by eating foods rich in folate. People who are homozygous for the C allele should maintain a healthy, balanced diet.

Possible Outcomes: Optimize Intake, Stay Balanced

Recommendations: Please also see the genetic test results for the related phenotypes: "Methotrexate toxicity" and "Genetic risk due to decreased vitamin B2".

Markers Tested and Scientific Strength: MTHFR [rs1801133]

The rs1801133 marker is rated a “3”.

“Scientific Strength” refers to the strength of research evidence for the genetic marker and the associated result. A rating of “4” indicates a study of over 2,000 people and at least one study that replicated the results. A rating of “3” indicates a study of over 400 people. A rating of “2” indicates a study of less than 400 people; studies in this category are preliminary but pass the criteria for statistical significance. A rating of “1” indicates that results are extremely preliminary.

Limitations and Warnings: This test reports on genetic predisposition for decreased folate levels. Other tests are available to assess a patient’s levels of blood folate. An ‘Optimize Intake’ genetic result does not indicate that the patient’s actual blood folate levels are too low, but rather that the patient may be genetically predisposed to have lower blood folate levels. Similarly, a ‘Stay Balanced’ genetic result does not indicate that the patient’s actual blood folate levels are optimal.

These interpretations and recommendations are made in the context of studies that included Caucasian, African and Hispanic participants, and the results may or may not be relevant to tested individuals who are of Asian ancestry.

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Metoprolol metabolism

(DC:TB-0217.001 06DEC2012)

*Report Type: Pharmacogenetics

About: Metoprolol (MET) is one of the most commonly used beta-blockers for treatment of heart failure.[1] This cardioselective beta(1)-adrenergic blocker is also used for treatment of acute myocardial infarction, angina pectoris and mild to moderate hypertension.[2] Metoprolol is metabolized to inactive forms by a member of the cytochrome P450 superfamily, the CYP2D6 enzyme, which is responsible for 70% to 80% of the metabolism of metoprolol.[3]

Genetics: CYP2D6 variants play a major role in the pharmacokinetics of metoprolol, which translates into relevant clinical outcomes.[3,4,5,6] A wide range of variants in the CYP2D6 gene affect the enzyme activity, and individuals can be classified based on this activity into three metabolizer groups: Extensive Metabolizer (normal enzyme activity), Intermediate Metabolizer (intermediate enzyme activity) and Poor Metabolizer (low or no enzyme activity).

CYP2D6 poor and intermediate metabolizers treated with standard doses of metoprolol have increased plasma concentration of the drug and increased risk for bradycardia.[7,8,9,10,11,12,13] They may also have greater reductions in heart rate, diastolic blood pressure, and arterial pressure at typical doses.[3,6,14,15,16,17,18]

The largest and most representative study on CYP2D6-metabolized beta-blockers demonstrates that the CYP2D6 poor metabolizer status is associated with lower heart rate and blood pressure.[3] The association between CYP2D6 genotype and metoprolol response or side effects was not confirmed in some much smaller studies.[7,11,19,20]

Recommendations: The FDA-approved labels of metoprolol[12,13] indicate that concomitant use of CYP2D6 inhibitors (see "Known CYP2D6 Inhibitors" table below) with metoprolol may lead to higher than normal plasma levels of metoprolol at standard doses. CYP2D6 inhibitors are also likely to decrease the cardioselectivity of metoprolol. The Royal Dutch Association for the Advancement of Pharmacy's Pharmacogenetics Working Group also suggests dose adjustment or alternative medications for CYP2D6 poor metabolizers and intermediate metabolizers.[21] CYP2D6 genotype and metabolizer status may also affect a patient's response to other drugs (see "Known CYP2D6 Substrates" table below).

Possible Outcomes: Poor Metabolizer, Intermediate Metabolizer, Extensive Metabolizer

Markers or Alleles Tested: CYP2D6 alleles are classified as non-functional (*3, *4, *6 and *8), reduced-function (*9, *10, *17, *29 and *41), and normal-function (*1, *2 and *35). CYP2D6 phenotype prediction follows conventional guidelines (See below).

Predicted CYP2D6 Metabolizer Status

CYP2D6 Diplotype	Predicted Metabolizer Status
Two non-functional alleles	Poor metabolizer
One non-functional allele plus one reduced-function allele, or two reduced-function alleles	Intermediate metabolizer
One or two normal-function copies of the CYP2D6 gene	Extensive metabolizer

Ethnic distribution of CYP2D6 phenotypes

Ethnicity	Poor Metabolizer	Intermediate Metabolizer	Extensive Metabolizer
African American	2-8%	~30%	60-70%
Caucasian	5-10%	10-17%	70-80%
East Asian	<2%	50-60%	40-50%
Hispanic	3-10%	no data	no data

Limitations and Warnings: This test detects common CYP2D6 alleles that have a combined frequency of over 90% in major ethnic groups. Other alleles that may also affect CYP2D6 enzyme activity are not tested. For example, the test does not include alleles that are associated with higher than normal enzyme activity. As such, a small percentage of patients with the test result of CYP2D6 "Extensive Metabolizer" may metabolize metoprolol at higher than normal rates. This may result in failure to achieve optimal plasma concentrations at standard doses and an increased risk of therapeutic failure.[4,7,16]

Known CYP2D6 Inhibitors and Substrates Tables

Known CYP2D6 Inhibitors[22,23,24]		
amiodarone	escitalopram	paroxetine
bupropion	flecainide acetate	perphenazine
celecoxib	fluoxetine	primaquine phosphate
chloroquine phosphate	fluphenazine	propafenone
chlorpheniramine	fluvoxamine maleate	propoxyphene
chlorpromazine	halofantrine	quinacrine
cimetidine	haloperidol	quinidine
cinacalcet	histamine H1 receptor antagonists	ranitidine
citalopram	hydroxychloroquine	reduced haloperidol
clemastine	hydroxyzine	ritonavir
clomipramine	labetalol	sertraline
cocaine	levomepromazine	terbinafine
codeine	lomustine	thioridazine
darifenacin hydrobromide	lumefantrine	ticlopidine
delavirdine mesylate	methadone	tripelennamine
desipramine	metoclopramide	valdecoxib
diphenhydramine	mibefradil	vinblastine sulfate
doxepin	midodrine	vincristine sulfate
doxorubicin	moclobemide	vinorelbine tartrate
dronedarone	norfluoxetine	yohimbine
duloxetine		

Known CYP2D6 Substrates[22,24,25]		
alprenolol	fenfluramine	oxycodone
amitriptyline	fentanyl	paliperidone
amoxapine	flecainide	paroxetine
amphetamine	fluoxetine	penbutolol sulfate
aripiprazole	fluphenazine	pentazocine lactate
atomoxetine	fluvoxamine	perhexiline
betaxolol	formoterol	perphenazine
bisoprolol	galantamine	phenacetin
bufuralol	haloperidol	phenformin
captopril	hydrocodone	pindolol
carvedilol	hydrocortisone	promethazine
cevimeline	hydroxyamphetamine hydrobromide	propafenone
chlorpheniramine	iloperidone	propoxyphene
chlorpromazine	imipramine	propranolol
chlorpropamide	labetalol	protriptyline
cinacalcet	lidocaine	quetiapine
clomipramine	loratadine	ranolazine
clonidine	maprotiline	risperidone
clozapine	meperidine	ropivacaine
codeine	methadone	selegiline
cyclobenzaprine	methamphetamine	S-metoprolol
cyclophosphamide	methoxyamphetamine	sparteine
darifenacin hydrobromide	metoclopramide	tamoxifen
debrisoquine	metoprolol	tamsulosin
delavirdine mesylate	mexiletine	thioridazine
desipramine	minaprine	tiagabine
dexfenfluramine	mirtazapine	timolol
dextromethorphan	molindone	tolterodine
diphenhydramine	morphine	tramadol
dolasetron	nebivolol	trazodone
donepezil	nortriptyline	trimipramine maleate
doxazosin mesylate	olanzapine	venlafaxine
doxepin	ondansetron	yohimbine
duloxetine	orphenadrine citrate	zuclopenthixol
encainide		

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
N/A	V58.83 Encounter for therapeutic drug monitoring

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Hypertension

Report Type: Health Conditions

About: The prevalence of hypertension, also known as high blood pressure, is estimated to be 29% in the U.S.[1] This condition can lead to stroke, heart attack and kidney failure. Risk factors for hypertension include high salt intake, being overweight and high alcohol consumption.[2] Research indicates that genetic factors are also associated with risk for the condition.[3,4,5,6]

Genetics: Over 90% of individuals who develop hypertension are considered to have essential or primary hypertension, meaning that no underlying medical cause can be identified. Hypertension can also be secondary to existing medical problems, such as kidney disease. Some rare forms of hypertension are caused by mutations in single genes. Individuals with these mutations usually have a family history of the disease and hypertension occurs at a younger age. This genetic test focuses on essential hypertension, for which several susceptibility alleles have been identified through large population studies.[3,4,5,6]

Because of the complex interactions of different physiologic pathways regulating blood pressure, it has been difficult to definitively identify risk alleles in candidate genes for hypertension. Contradictory results have been reported for many genes identified in an early study but not replicated in further research. A few large population-based genome-wide scans and candidate gene association studies have identified two genes as risk factors for hypertension: PPARGC1A and BCAT1.[3,4,5,6]

The PPARGC1A gene encodes the protein PPAR-gamma coactivator 1,[7] which regulates the expression of genes involved in several metabolic processes related to blood pressure homeostasis. The Gly482Ser (rs7961152) allele causes an amino acid change in the protein, but the functional consequence of this change is unknown. The Ser allele was associated with decreased risk for essential hypertension in a Danish population.[5] The same allele was also modestly associated with decreased risk for severe essential hypertension in a Chinese population.[6]

The BCAT1 gene encodes branched-chain aminotransferase 1, which catalyzes the first reaction in the catabolism of the essential branched-chain amino acids leucine, isoleucine and valine. The rs7961152 marker is in a non-coding region of the BCAT1 gene, making it difficult to discern the functional impact of a nucleotide change. The variant may be linked to another causative mutation in the same gene or a neighboring gene. The A allele of the rs7961152 marker was associated with an increased risk for essential hypertension in a study of a British Caucasian population.[4] In a Korean population, the same allele was moderately associated with increased blood pressure.[3]

Recommendations: The U.S. Preventive Services Task Force recommends screening for high blood pressure in adults aged 18 and older.[8]

Possible Outcomes: Increased Risk, Above Average Risk, Average Risk

Markers Tested

Gene/ Locus[a]	Marker[b]	Associated Allele[c]	Odds Ratio[d]	Ethnicity[e]	Population Frequency[f]	Scientific Strength[g]	PMID[h]
BCAT1	rs7961152	A	1.29	Asian	16.9%	Preliminary	19424278
BCAT1	rs7961152	A	1.16	Caucasian	46.5%	Preliminary	17554300
PPARGC1A	rs8192678	A	0.60	Asian	40.5%	Preliminary	17971240
PPARGC1A	rs8192678	A	0.70	Caucasian	35.0%	Preliminary	15738346

[a]Gene or locus containing the tested marker

[b]Marker tested

[c]Allele associated with disease risk

[d]Measure of the likelihood that an individual will get the disease if carrying a specific allele

[e]Ethnicity of the population in the corresponding study

[f]Percentage of people who have the associated allele in the population studied

[g]Validated markers represent the highest quality genetic markers available; preliminary markers represent the latest in genetic research and have not met our highest standards for validation.

[h]PubMed is a service managed by the National Library of Medicine; thePubMed ID (PMID) number identifies the referenced study.

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
401.1 Benign essential hypertension	N/A

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Beta-blockers, LVEF response

(DC:TB-0208.002 07MAY2013)

Report Type: Pharmacogenetics

About: Beta-blockers (carvedilol, metoprolol, etc.) are used as a standard therapy for heart failure.[1] This class of drugs antagonizes beta-adrenergic receptors and down-regulates the effects of catecholamine hormones (epinephrine and norepinephrine). Genetic variants that affect beta-adrenergic signaling may influence outcomes in heart failure patients treated with beta-blockers.

Genetics: In heart failure patients, the benefits of treatment with beta-blockers for left ventricular ejection fraction (LVEF) are associated with variants in the ADRB1 gene, which encodes the beta(1)-adrenergic receptor.[2] A meta-analysis of three pharmacogenetic studies in heart failure patients found that individuals who are homozygous for the Arg389 allele have a greater increase in LVEF in response to beta-blockers (carvedilol, metoprolol or bisoprolol) compared to individuals who carry the Gly389 allele.[2] This genetic effect was also reported in a study involving patients who underwent 1.5 years of carvedilol treatment.[3]

In a number of small studies, the Arg389 allele has been studied in relation to other phenotypes, including exercise heart rate, response to rate-control therapy in atrial fibrillation, changes in heart rate and blood pressure in patients with essential hypertension and LV hypertrophy, and transplant-free survival in heart failure patients.[4,5,6,7] However, it is still controversial whether Arg389 modifies the outcomes of beta-blocker therapy. Some studies showed no association, while others reported significant associations but do not always agree on the direction of the genetic effect.

Recommendations: NA

Possible Outcomes: Enhanced Benefit, Beneficial

Markers or Alleles Tested: ADRB1 [rs1801253]

Ethnic Distribution of Tested Alleles:

Ethnicity	Arg389 allele (C allele) frequency[8]
Caucasian	~70%
East Asians	75-85%
Africans	~60%

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test,

nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
995.29 Unspecified adverse effect of other drug, medicinal and biological substance Applies to: • Unspecified adverse effect of medicinal substance NEC properly administered	N/A

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Myocardial infarction

Report Type: Health Conditions

About: Myocardial infarction (MI) kills approximately half a million people in the U.S. each year.[1] Symptoms of this condition include chest pain, shortness of breath and other symptoms.[1,2] Risk factors include a family history of MI, diabetes, hypertension and hypercholesteremia.[2] Research indicates that genetic factors are also associated with MI.[2,3]

Genetics: Genetic susceptibility factors contribute to the risk of MI. Their importance is highlighted by the fact that about 15% to 20% of individuals who have an MI lack conventional risk factors.[4] Studies suggest that many genetic variations associated with the disease are in genes involved in processes involving endothelial cell function, inflammation, lipid metabolism, thrombosis and fibrinolysis.[2,3]

Many SNPs associated with heart disease are in genes involved in inflammation. A marker in the CXCL12 gene, which encodes a chemokine molecule important for attracting lymphocytes,[5] was shown to be also associated with the risk for MI in a large study of Caucasians.[6] Additionally, in a large genome-wide association study identifying markers affecting eosinophil counts, a marker at 12q24 (rs3184504) in the SH2B3 gene, which encodes a protein important for cytokine pathway inhibition, was associated with risk for MI in six different populations.[7]

A number of high-impact studies have identified 9p21.3 as an important genetic region associated with risk for MI or coronary artery disease.[8] This region contains no annotated genes but lies close to the CDKN2A and CDKN2B genes, which regulate important cell cycle pathways. An allele of the rs10757278 marker in the 9p21.3 region is associated with MI in Icelandic, U.S. Caucasian, and Italian populations.[9,10,11] When the Italian patient group was subdivided into those with and without a family history of MI, only the individuals with a family history of MI still showed a significant association with the allele.[11] This research suggests that in cases with a family history of heart disease and heart attack, the relative risk for an individual carrying a risk allele can be significantly higher.

Another large study carried out by the Myocardial Infarction Genetics Consortium replicated the region of 9p21, as well as identified or replicated other markers in MIA3 (rs17465637), 1p13 (rs646776), 10q11 in CXCL12 (rs1746048), 21q22 (rs9982601), 6p24 in PHACTR1 (rs12526453), and 2q33 in WDR12 (rs6725887).[6]

Recommendations: NA

Possible Outcomes: Increased Risk, Above Average Risk, Average Risk

Markers Tested

Gene/Locus[a]	Marker[b]	Associated Allele[c]	Odds Ratio[d]	Ethnicity[e]	Population Frequency[f]	Scientific Strength[g]	PMID[h]
PSMA6	rs1048990	G	1.21	Asian	37.8%	Validated	16845397
Intergenic_9p21	rs10757278	G	1.28	Caucasian	50.0%	Validated	17478679
PCSK9	rs11206510	T	1.15	Caucasian	83.6%	Validated	19198609
PHACTR1	rs12526453	C	1.12	Caucasian	63.3%	Validated	19198609
CXCL12	rs1746048	C	1.17	Caucasian	85.4%	Validated	19198609
MIA3	rs17465637	C	1.14	Caucasian	26.8%	Validated	19198609
SH2B3	rs3184504	T	1.13	Caucasian	44.5%	Validated	19198610
Intergenic_1p13	rs646776	T[i]	1.19	Caucasian	74.6%	Validated	19198609
WDR12	rs6725887	C	1.17	Caucasian	15.9%	Validated	19198609
LGALS2	rs7291467	C[j]	1.23	Asian	73.3%	Validated	15129282
Intergenic_21q22	rs9982601	T	1.20	Caucasian	20.6%	Validated	19198609
LTA	rs1041981	A	1.78	Asian	43.5%	Preliminary	12426569
OR13G1	rs1151640	G	1.31	Caucasian	46.0%	Preliminary	16175505
PRR4	rs1376251	C	1.23	Caucasian	65.3%	Preliminary	16175505
MIAT	rs2331291	T	1.38	Asian	13.1%	Preliminary	17066261

[a]Gene or locus containing the tested marker.

[b]Marker tested.

[c]Allele associated with disease risk.

[d]Measure of the likelihood that an individual will get the disease if carrying a specific allele.

[e]Ethnicity of the population in the corresponding study.

[f]Percentage of people who have the associated allele in the population studied.

[g]Validated markers represent the highest quality genetic markers available; preliminary markers represent the latest in genetic research and have not met our highest standards for validation.

[h]PubMed is a service managed by the National Library of Medicine; the PubMed ID (PMID) number identifies the referenced study.

[i]This marker can be assayed on either strand of DNA. Therefore, the associated allele could be reported as either an A or a T in the patient report.

[j]This marker can be assayed on either strand of DNA. Therefore, the associated allele could be reported as either a C or a G in the patient report.

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
410.9 Acute myocardial infarction of unspecified site Applies to: • Acute myocardial infarction NOS • Coronary occlusion NOS • Myocardial infarction NOS	V81.2 Screening for other and unspecified cardiovascular conditions

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Perindopril

(DC:TB-0236.001 06DEC2012)

Report Type: Pharmacogenetics

About: Perindopril is an angiotensin-converting enzyme (ACE) inhibitor that is used to manage or treat hypertension, stable coronary artery disease, myocardial infarction and heart failure.[1,2] ACE is a zinc metallopeptidase that plays a role in the production of angiotensin II, the primary blood pressure effector in the renin-angiotensin-aldosterone system.[3] It also acts in the degradation of bradykinin, a strong vasodilator of the Kinin-Kallikrein cascade.[4] By preventing the production of angiotensin II and blocking the degradation of bradykinin, ACE inhibitors prevent arteriolar vasoconstriction and promote vasodilation.[5]

Genetics: Two markers located in the angiotensin II receptor type I (AGTR1) gene and one in the bradykinin type I receptor (BDKRB1) gene are associated with perindopril's treatment benefit. The benefit of perindopril in stable coronary artery disease patients is calculated using these three markers to generate a pharmacogenetic score.[6]

In a study that examined the effect of perindopril (8 mg/day) in predominantly Caucasian male patients with stable coronary artery disease, markers in AGTR1 (rs275651 and rs5182) and BDKRB1 (rs12050217) were associated with the treatment benefits of perindopril. Combining these three markers in a pharmacogenetic score demonstrated a stepwise decrease in treatment benefit of perindopril. A pronounced treatment benefit was observed in a subgroup of 73.5% of patients, whereas no benefit was apparent in the remaining 26.5%, with a trend towards a harmful effect.[6] Prospective studies are yet to be completed to test whether patients with a "non-responder" genetic profile should avoid treatment with ACE inhibitors.[7]

Recommendations: NA

Possible Outcomes: Likely Non-Responder, Likely Responder

Markers or Alleles Tested: AGTR1 [rs275651, rs5182]; BDKRB1 [rs12050217]

Limitations and Warnings: This test can inform perindopril treatment optimization for stable coronary artery disease patients only. The results of this test do not apply to patients that are being treated with perindopril for other indications.

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
995.29 Unspecified adverse effect of other drug, medicinal and biological substance Applies to: • Unspecified adverse effect of medicinal substance NEC properly administered	N/A

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Peripheral arterial disease

Report Type: Health Conditions

About: Peripheral arterial disease (PAD) affects 20% of individuals in the U.S. over the age of 55 and is strongly associated with a risk for myocardial infarction and stroke.[1] This progressive disease is characterized by the accumulation of plaque in the arterial system, especially the abdominal aorta and arteries in the legs.[1,2] Risk factors for PAD include age, sex, smoking, diabetes, hypertension and hyperlipidemia.[1] Research indicates that genetic factors are also associated with PAD.[3]

Genetics: Peripheral arterial disease (PAD) is caused by atherosclerosis, a complex disorder involving both genetic risk factors as well as traditional risk factors, such as cigarette smoking, cholesterol levels, hypertension and stress. Within various populations, genetic differences are thought to account for greater than 50% of the risk for atherosclerosis.[3] It is estimated that the genetic risk of atherosclerosis involves variants in hundreds of genes with a variety of functions in regulating blood pressure, lipid and cholesterol metabolism, pro-inflammatory processes, cell adhesion and migration.[3]

The CHRNA3 gene encodes a subunit of the nicotinic acetylcholine receptor, which binds to nicotine.[4] A large study of PAD in Caucasians from Iceland, New Zealand, Austria, Sweden and Italy[5] showed that a variant in CHRNA3 increased the risk for PAD by approximately 20%. No significant differences in risk were shown between males and females. The results from this study suggest that this variant may also partly confer a risk for cardiovascular disease indirectly through its relationship with exposure to nicotine as a function of smoking history.

Recommendations: NA

Possible Outcomes: Increased Risk, Above Average Risk, Average Risk

Marker Tested

Gene/ Locus[a]	Marker[b]	Associated Allele[c]	Odds Ratio[d]	Ethnicity[e]	Population Frequency[f]	Scientific Strength[g]	PMID[h]
CHRNA3	rs1051730	T	1.19	Caucasian	38.5%	Validated	18385739

[a]Gene or locus containing the tested marker

[b]Marker tested

[c]Allele associated with disease risk

[d]Measure of the likelihood that an individual will get the disease if carrying a specific allele

[e]Ethnicity of the population in the corresponding study

[f]Percentage of people who have the associated allele in the population studied

[g]Validated markers represent the highest quality genetic markers available; preliminary markers represent the latest in

genetic research and have not met our highest standards for validation.

[h]PubMed is a service managed by the National Library of Medicine; thePubMed ID (PMID) number identifies the referenced study.

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
443.9 Peripheral vascular disease, unspecified Applies to: • Intermittent claudication NOS • Peripheral: ◦ angiopathy NOS ◦ vascular disease NOS • Spasm of artery	V81.2 Screening for other and unspecified cardiovascular conditions

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Sickle cell anemia

Report Type: Carrier Status

About: Sickle cell anemia (also known as homozygous sickle cell disease or HbSS) is the most common inherited blood disorder in the U.S., accounting for 60% to 70% of all sickle cell disease in the country. The condition is a chronic, lifelong, inherited disorder that is associated with a decreased lifespan and characterized by the clumping of red blood cells, which can cause painful episodes and permanent damage to the eyes, brain, heart, lungs, kidneys, liver, bones and spleen. Infections and lung disease are the leading causes of death for people with sickle cell anemia.[1,2,3]

Genetics: Sickle cell anemia is caused by a mutation in the HBB gene, which encodes the beta subunit of hemoglobin. Individuals with two copies of the HbS mutation have sickle cell anemia, whereas individuals with one copy of the HbS mutation have sickle cell trait and can pass this mutation to their children.[1,4] Most people with sickle cell trait lead normal lives, but they are at increased risk for exertional rhabdomyolysis, a condition that may lead to exercise-induced sudden cardiac death.[5,6,7]

Sickle cell anemia is a hemoglobinopathy. In some individuals, the sickele cell mutation in HBB will occur along with other mutations in the HBB gene, including hemoglobin (HbC), hemoglobin E (HbE) and beta-thalassemia, complicating and increasing the severity of these hemoglobinopathies..[1]

Sickle cell anemia occurs in 1 in 600 to 700 African American births and in 1 in 19,000 to 46,000 Hispanic American births.[8]

Mode of Inheritance: Autosomal Recessive

Possible Outcomes: Not a Carrier, Carrier, Homozygote

Recommendations: The American College of Obstetricians and Gynecologists (ACOG) recommends offering carrier screening for hemoglobinopathies to people of African, Southeast Asian and Mediterranean ancestry.[9] The American College of Medical Genetics identified sickle cell anemia as one of 29 diseases for which newborn screening should be mandated.[10]

The NCAA requires that all Division I athletes undergo testing for sickle cell trait.[11] Individuals with sickle cell trait can compete and play sports at all levels as long as they are aware of the trait and take proper precautions.[12]

Mutations Tested: The test includes one mutation in the HBB gene.

HBB [hemoglobin S]

Ethnic Prevalence and Frequency of Mutation Tested

Gene	Population	Detection Rate[a]	Pre-Test Carrier Rate[b]	Post-Test Residual Risk[c]
HBB	African American	100%	1 in 15	0
HBB	Native American	100%	1 in 150	0
HBB	Hispanic American	100%	1 in 203	0
HBB	Middle Eastern	100%	1 in 478	0
HBB	Caucasian	100%	1 in 642	0
HBB	Asian Indian	100%	1 in 652	0
HBB	Flilipino	100%	1 in 879	0
HBB	Asian	100%	1 in 1,315	0
HBB	Southeast Asian	100%	1 in 2,365	0

[a]Percentage of affected patients carrying mutations detected by this test.

[b]Carrier risk before testing.[13]

[c]Carrier risk after a “not a carrier” test result.

Limitations and Warnings: Sickle cell anemia carriers may have misleading results in the hemoglobin A1C test (also known as glycated hemoglobin, glycosylated hemoglobin, and HbA1c) that is used to diagnose diabetes and to monitor blood glucose levels in diabetics. False A1C test results can lead to false diagnosis, over-treatment or under-treatment of diabetes in people with hemoglobin variants.[14]

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
282.60 Sickle-cell disease, unspecified Applies to: • Sickle-cell anemia NOS	V78.2 Screening for sickle cell disease/trait

References

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Simvastatin-induced myopathy

(DC:TB-0229.001 06DEC2012)

Report Type: Pharmacogenetics

About: Simvastatin is a member of the statins, a class of cholesterol-lowering drugs whose major adverse effect is skeletal muscle toxicity. Approximately 5% to 10% of patients taking statins experience muscle pain (myalgia).[1] A small portion of patients, (1.5% to 5.0%) may develop more severe symptoms indicating muscle degradation (myopathy).[1] In rare cases (0.1 to 0.2 cases per 1,000 person-years), severe muscle damage leads to acute, potentially lethal kidney failure (rhabdomyolysis).[1,2]

Genetics: Simvastatin-induced myopathy has been shown to be influenced by variation in the SLCO1B1 gene.[3] Approximately 60% of myopathy cases in a simvastatin (80 mg/day) clinical trial were attributed to the C allele of the rs4149056 marker in the SLCO1B1 gene.[3] SLCO1B1 encodes the organic anion-transporting polypeptide 1B1 (OATP1B1, also known as OATP-C or OATP2), which regulates the hepatic uptake of statins and other drugs. The C allele at rs4149056 reduces the activity of the OATP1B1 transporter,[4] leading to increased blood simvastatin levels and the potential for increased toxicity to the muscles. However, available clinical data are insufficient to show whether the SLCO1B1 variant also alters myopathy risk associated with the use of statins other than simvastatin.[5]

The risk of myopathy varies with the type of statin and is dose-related. Some statins are associated with lower risk of myopathy compared with others,[1] and the pharmacokinetic effects of variants of rs4149056 are not uniform for different statins.[6,7] The incidences of myopathy and rhabdomyolysis while taking 80 mg simvastatin daily are disproportionally higher than those with lower doses.[8]

Genetic variation in SLCO1B1 also affects pharmacokinetics of other drugs, such as methotrexate and HIV protease inhibitors.[9]

Recommendations: The NIH Clinical Pharmacogenetics Implementation Consortium (CPIC) published guidelines for SLCO1B1 genotyping and simvastatin-induced myopathy, recommending reduced dose or alternative statins for patients with the C allele at rs4149056. The CPIC also recommends routine surveillance of serum creatine kinase levels for those patients.[5]

Possible Outcomes: Increased Risk, Typical Risk

Markers and Alleles Tested: SLCO1B1 [rs4149056]

Ethnic Distribution of Tested Allele

Frequency of C allele of the rs4149056 marker in major ethnic groups.[5]

Ethnicity	C allele frequency (rs4149056)
Caucasian	15%
African	3%
Middle Eastern	20%
Asian	13%

Limitations and Warnings: Although the tested SLCO1B1 variant has the most significant genetic effect on the risk of simvastatin-induced myopathy in clinical studies, rarer mutations in SLCO1B1 that may also affect the function of the encoded protein are not screened in this test. Current knowledge is limited on the involvement of other genes in the metabolism and clinical effects of simvastatin. In addition to genetic effects, the risk of simvastatin-induced myopathy varies with the patient's age, gender, body mass index, ethnicity and other clinical factors.[5]

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
359.4 Toxic myopathy	N/A

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Venous thrombosis

Report Type: Health Condition

About: Venous thrombosis is the formation of a blood clot in the veins that can potentially lead to thromboembolism. The individual risk of venous thromboembolism (VTE) is determined by a complex interaction of genetic, circumstantial and environmental factors. Risk factors include immobility, surgery, trauma, cancer, hormonal therapy, pregnancy, advanced age and family history.[1,2] Genetic factors are also associated with the risk of VTE development.

Genetics: Factor V Leiden, a mutation in the F5 gene, is the most common and most studied genetic prothrombotic defect, with an overall prevalence in Caucasians of approximately 5%. It is found in 20% of all patients with venous thrombosis, and in up to 50% of patients with thrombophilia.[3] The F5 gene encodes coagulation factor V, an important cofactor that accelerates the activation of prothrombin to thrombin in the blood coagulation cascade. The Factor V Leiden mutation impairs down-regulation of coagulation factor V, resulting in increased risk of clotting.

Individuals who are heterozygous for the Factor V Leiden mutation have a 3.5-fold increase in risk of VTE.[1,4,5,6] Homozygous patients are at a higher risk than heterozygous patients.[1,4]

After Factor V Leiden, the most common mutation associated with heritable thrombophilia is prothrombin G20210A,[7] which is located in the 3'-untranslated region of the gene and is associated with increased levels of prothrombin.[3] Increased levels of prothrombin are a risk factor for thrombosis.[8] Individuals with the mutation are at increased risk of VTE,[1,4,5,6] and risk further increases in individuals who have the Factor V Leiden mutation.[9,10] Individuals who are heterozygous for both mutations have a 20-fold increase in risk, whereas individuals who are heterozygous for either mutation only have a four- to five-fold increase in VTE risk.[11]

Among women with history of VTE, the Factor V Leiden and prothrombin G20210A mutations are independently associated with increased risk of VTE during pregnancy and puerperium.[10] Risk of thrombosis increases more than 100-fold in pregnant women who have both mutations compared to women without the mutations.[10,12]

MTHFR is an important enzyme in folate metabolism and DNA synthesis. The homozygous MTHFR C677T genotype has been associated with an increased risk of elevated plasma homocysteine levels[13] and hyperhomocysteinemia, an independent risk factor for VTE.[14,15,16] The homozygous C677T genotype has also been associated with risk of VTE in Chinese and Korean populations.[5,14,17,18] A meta-analysis found that, in studies of non-Americans, the homozygous C677T genotype was associated with a 20% higher risk of VTE compared to the homozygous wild-type genotype.[16] In contrast, the homozygous C677T genotype had no effect on VTE in North America, possibly due to the higher intake rates of folate and riboflavin. In support of this hypothesis, one study found that homocysteine levels in homozygous C677T individuals were significantly higher than in homozygous wild-type individuals only if folate levels were below 15.4 nmol/L.[15,19] Thus, individuals who are homozygous for C677T may require more dietary folate than individuals who are wild-type.

Recommendations: The American College of Medical Genetics, the American College of Obstetricians and Gynecologists and the European International Thrombophilia Guidelines recommend Factor V Leiden and/or G20210A testing in populations that are likely to have a mutation.[7,20,21]

Possible Outcomes: Increased Risk, Above Average Risk, Average Risk

Markers and Alleles Tested: F5 [Factor V Leiden/R506Q]; F2 [G20210A]; MTHFR [C677T]

Ethnic Distribution of Tested Alleles: The Factor V Leiden and prothrombin G20210A mutations are common in Caucasians but extremely rare in Asians and Africans.[22] The allele frequency of Factor V Leiden in the U.S. population is 5% in Caucasians, 2.2% in Hispanics and 1.2% in blacks.[1,23] Prothrombin G20210A has a prevalence of approximately 2% in the US population and occurs primarily in Caucasians.[1] Double heterozygosity for Factor V Leiden and prothrombin G20210A is estimated to affect 1 in 1,000 individuals in the general population.

There is significant ethnic and geographic variation in the frequency of C677T. The prevalence of the homozygous C677T genotype ranges from around 1% in Black populations in the US, sub-Saharan Africa, and South America to more than 20% in US Hispanics, Colombians and Amerindians in Brazil. The homozygous C677T genotype occurs at a frequency of 8-20% in Caucasians in Europe, North America, and Australia and at 12% in Japanese.[24]

Limitations and Warnings: Genetic variants in other proteins, such as protein S, protein C and antithrombin are known to increase the risk of venous thrombosis, but are not part of this test. Non-genetic factors known to increase the risk of venous thrombosis include age, obesity, trauma/surgery, smoking, pregnancy and airplane travel.[25]

According to the American College of Medical Genetics (ACMG) Consensus Statement on Factor V Leiden Mutation Testing, the MTHFR C677T only accounts for a third of hyperhomocysteinemia cases, and plasma measurements of homocysteine may be more informative than molecular methods.[7]

Dietary factors, such as folic acid intake, may influence the association between MTHFR and VTE.[19]

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
453.9 Other venous embolism and thrombosis of unspecified site • Embolism of vein • Thrombosis (vein)	N/A

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Verapamil and QTc interval

(DC:TB-0235.001 06DEC2012)

Report Type: Pharmacogenetics

About: Verapamil is an L-type calcium-channel blocker that is used to treat hypertension and angina. Its interaction with calcium channels reduces the force and rate of muscle contraction, leading to decreased blood pressure and heart rate.[1]

Genetics: The nitric oxide synthase 1 (neuronal) adaptor protein, which is encoded by the NOS1AP gene, regulates the synthesis of nitric oxide, a known regulator of intracellular calcium.[2,3] Variants in the NOS1AP gene are associated with the prolongation of QTc interval in patients taking verapamil.[4]

When treated with verapamil, patients who were homozygous for the G allele at the tested marker in NOS1AP were more likely to display verapamil-induced QTc interval prolongation, compared to individuals who were not.[4] Individuals of any genotype on other calcium-channel blocker medications did not show this potentiation effect.[4] The association of this NOS1AP marker with verapamil-induced QTc interval prolongation has not yet been independently replicated. In some studies, a prolonged QTc has been shown to increase the risk for developing certain tachyarrhythmias that increase the risk of sudden cardiac death.[5,6] However, QTc interval length depends on many factors, including age, gender and other genes. In addition, QTc interval prolongation has been associated with certain medications and specific disease pathologies.[7,8]

Recommendations: NA

Possible Outcomes: Increased Risk of Prolongation, Typical Risk of Prolongation

Markers or Alleles Tested: NOS1AP [rs10494366]

Ethnic Distribution of Tested Alleles: NA

Limitations and Warnings: NA

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
995.29 Unspecified adverse effect of other drug, medicinal and biological substance Applies to: • Unspecified adverse effect of medicinal substance NEC properly administered	N/A

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Verapamil vs. atenolol

(DC:TB-0232.001 06DEC2012)

Report Type: Pharmacogenetics

About: Verapamil is an L-type calcium-channel blocker that is used to treat hypertension and angina. Its interaction with calcium channels reduces the force and rate of muscle contraction, leading to decreased blood pressure and heart rate.[1]

Atenolol is a cardioselective beta(1)-adrenergic antagonist that is used to manage hypertension and chest pain caused by coronary heart disease. By inhibiting beta(1)-adrenergic receptors in the heart and smooth muscle of the vasculature, atenolol decreases resting heart rate, cardiac output and blood pressure.[2]

Genetics: Calcium-channel blockers, such as verapamil, bind to L-type calcium channels via the alpha1c-subunit, which is a major component of the channel pore and is encoded by the CACNA1C gene. Variants in CACNA1C are associated with a patient's response to different treatment strategies (beta-blockers versus calcium-channel blockers), but the mechanism is unknown.[3] Variants in CACNA1C have also been shown to be associated with blood pressure[4] and a reduction in blood pressure in response to calcium-channel blockers.[5,6]

Based on a study that included patients with hypertension and stable coronary artery disease, those individuals who were homozygous for the A allele at the tested marker in CACNA1C and were treated with verapamil were more likely to have decreased incidence of death, myocardial infarction or stroke compared to individuals with the same genotype who were treated with the beta-blocker atenolol. On the other hand, patients who were homozygous for the G allele and were treated with atenolol were more likely to have decreased incidence of death, myocardial infarction or stroke compared to individuals with the same genotype who were treated with verapamil. For heterozygous patients, no association of increased benefits with atenolol versus verapamil was found. It is important to note that all patients in this study had their blood pressure under control regardless of medication taken. Consequently, the mechanism that explains how different genotypes lead to the different outcomes in response to calcium-channel blockers and beta-blockers is not understood.[3] The association of this CACNA1C marker with benefits on verapamil or atenolol has not yet been independently replicated.

Recommendations: NA

Possible Outcomes: Likely Increased Benefit on Atenolol, Likely Increased Benefit on Verapamil, Likely Similar Benefit on Verapamil or Atenolol

Markers or Alleles Tested: CACNA1C [rs1051375]

Ethnic Distribution of Tested Alleles: NA

Limitations and Warnings: The results of this test are based on a study of patients with hypertenstion and stable coronary artery disease. Thus, they may not apply to patients being treated with verapamil or atenolol for other conditions.

Compatible ICD-9 Codes: Insurance coverage is not required for genetic testing. The diagnostic code (ICD-9) information provided herein is for insurance information purposes only and does not guarantee insurance coverage for any genetic test, nor is it intended to be a definitive list of diagnosis codes that may be applicable for any individual patient. The testing laboratory will pursue reimbursement directly from the patient should the patient's insurance carrier refuse to provide coverage.

Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
995.29 Unspecified adverse effect of other drug, medicinal and biological substance Applies to: • Unspecified adverse effect of medicinal substance NEC properly administered	N/A

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Warfarin

(DC:TB-0230.002 31JUL2013)

Report Type: Pharmacogenetics

About: Warfarin is the most frequently used oral anticoagulant worldwide, prescribed for prophylaxis and treatment of thrombotic disorders and thromboembolic events. Such indications include venous thrombosis, pulmonary embolism, atrial fibrillation and cardiac valve replacement. Warfarin is highly efficacious, but its narrow therapeutic index and large interindividual dosing variability lead to a high incidence of adverse events. Improper warfarin dosing is the second leading cause of drug-related emergency room visitation[1] and the number one cited reason for drug-related mortality.[2]

Warfarin acts as an anticoagulant through its ability to inhibit reduction of vitamin K by the vitamin K epoxide reductase complex subunit 1 (VKORC1) enzyme complex. Reduced vitamin K is an essential cofactor of gamma-glutamyl carboxylase, the enzyme that activates coagulation factors II, VII, IX and X. By decreasing the amount of reduced vitamin K available, warfarin depresses the activation of factors II, VII, IX and X into functional, coagulant proteins, and, therefore, decreases the ability of blood to clot. The primary metabolizing enzyme of warfarin is cytochrome P450 2C9 (CYP2C9).[3]

In 2010, the United States Food and Drug Administration (FDA) released a table of dosing recommendations for initiation of warfarin therapy based on VKORC1 and CYP2C9 genotypes. This pharmacogenetics-based dosing table significantly improved accuracy of therapeutic dose prediction compared to the traditional strategy of empirically determined dose.[4]

Genetics: The A allele of the -1639G>A mutation in the VKORC1 gene has been shown to decrease hepatic expression of VKORC1 and, therefore, increase patient sensitivity to warfarin.[5,6,7] Research studies have shown that the therapeutic dose of warfarin in patients with two copies of the A allele was less than the dose of patients with two copies of the G allele, with a difference up to 2.0 to 4.5-fold.[7,8,9]

Individuals carrying *2 or *3 genetic variants of CYP2C9 clear warfarin at a 30% to 50% or 80% to 90% slower rate, respectively, and exhibit increased serum levels of warfarin compared to carriers of only the reference wild-type variant *1.[10,11] CYP2C9*2 and CYP2C9*3 variants may decrease the dose required for effective anticoagulation and may increase the time necessary to achieve stable, therapeutic effect.[9,12] The CYP2C9*6 variant may also reduce metabolic activity and the dose required for effective anticoagulation.[11,13]

Customizing initial warfarin dose to VKORC1 and CYP2C9 genotypes may decrease patient risk of bleeding complications and may reduce the time required to achieve a stable, therapeutic effect.[12,14,15]

Recommendations: The FDA-approved label for warfarin recommends initial dosing based on VKORC1 and CYP2C9 genotypes in addition to clinical factors.[16] The National Institutes of Health (NIH) Clinical Pharmacogenetics Implementation Consortium guidelines recommend initial dosing based on VKORC1 and CYP2C9 genotypes.[11]

Standard doses of warfarin may cause bleeding complications in patients whose genotypes indicate increased or substantially increased sensitivity to warfarin. These patients may require lower initial doses of warfarin. Increased laboratory monitoring may be appropriate.

Classes of drugs that potentially interact with warfarin include the following:[16] inhibitors or inducers of CYP2C9, CYP1A2 and/or CYP3A4, anticoagulants, antiplatelet agents, nonsteroidal anti-inflammatory agents, serotonin reuptake inhibitors, antibiotics, antifungals, and botanical (herbal) products and foods. This list is not complete. Consult the warfarin drug label[16] and the labels of all concurrently used drugs for more specifics about warfarin drug interactions.

Possible Outcomes: Substantially Increased Sensitivity, Increased Sensitivity, Typical Sensitivity

Markers or Alleles Tested: VKORC1 -1639G>A [rs9923231]; CYP2C9 [CYP2C9*2/rs1799853, CYP2C9*3/rs1057910, CYP2C9*6/rs9332131]

Ethnic Distribution of Tested Alleles

Frequency of VKORC1 and CYP2C9 alleles differs significantly between racial and ethnic groups.[17]

Gene	Allele	Caucasian	African American	Asian	Hispanic
VKORC1	-1639: G	59.4%	89.2%	33.3%	56.4%
VKORC1	-1639: A	40.6%	10.8%	66.7%	43.6%
CYP2C9	*1	78.8%	86.7%	92.2%	82.2%
CYP2C9	*2	15.1%	2.8%	2.9%	6.9%
CYP2C9	*3	5.7%	2.0%	3.9%	6.4%
CYP2C9	*6	0.0%	1.0%	0.0%	0.5%

Predicted Warfarin Sensitivity Status

	VKORC1 -1639G>A genotype		
CYP2C9 genotype (below)	G/G	G/A	A/A
*1/*1	Typical sensitivity	Typical sensitivity	Increased sensitivity
*1/*2	Typical sensitivity	Increased sensitivity	Increased sensitivity
*1/*3	Increased sensitivity	Increased sensitivity	Substantially increased sensitivity
*1/*6	Increased sensitivity	Increased sensitivity	Substantially increased sensitivity
*2/*2	Increased sensitivity	Increased sensitivity	Substantially increased sensitivity
*2/*3	Increased sensitivity	Substantially increased sensitivity	Substantially increased sensitivity
*2/*6	Increased sensitivity	Substantially increased sensitivity	Substantially increased sensitivity
*3/*3	Substantially increased sensitivity	Substantially increased sensitivity	Substantially increased sensitivity
*3/*6	Substantially increased sensitivity	Substantially increased sensitivity	Substantially increased sensitivity
*6/*6	Substantially increased sensitivity	Substantially increased sensitivity	Substantially increased sensitivity

Limitations and Warnings: Warfarin can cause major or fatal bleeding. Additional genetic variants within VKORC1, CYP2C9, and other genes not included in this test are known to affect warfarin sensitivity. Not all genetic factors influencing warfarin sensitivity have been identified. Regular monitoring of INR (international normalized ratio) should be performed on all treated patients.

Not all factors influencing warfarin response are known. Important non-genetic factors include age, sex, weight, height, race, ethnicity, comorbidities, warfarin indication, target INR, and use of tobacco and interacting medications.[11]

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Primary ICD-9 Code(s)	Screening ICD-9 Code(s)
E934.2 Anticoagulants causing adverse effects in therapeutic use	N/A

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