Estrogenomic Profile

GENOVATIONS™

63 Zillicoa Street
Asheville, NC 28801

Patient: SAMPLE
PATIENT
DOB:
Sex:
MRN:

Apo E

Location:
Chromosome 19
APOE
APO E2: cys / cys
APO E3: cys / arg
APO E4: arg / arg
Your Genotype:

3	3

The two SNPs lead to 3 possible variants for each chromosome, known as ApoE2, E3, & E4.

Apolipoprotein E : CHOLESTEROL REGULATION

Apolipoprotein E (Apo E) plays a key role in lipid metabolism by helping to remove dietary cholesterol (chylomicrons and VLDL) from the bloodstream.

Health Implications

- This genotype is the most common (accounting for >50% of most populations) and is the genotype against which E2 and E4 are compared
- APO E3 confers only a moderate tendency toward elevated total- and LDL cholesterol, and lower HDL-C
- Risk is intermediate between E2 and E4 for atherosclerosis, myocardial infarction, stroke (in smokers), and osteoporosis

Treatment Options

- Effects of cholesterol and dietary fat on serum cholesterol levels is least with the E2 allele and greatest with the E4 allele; thus, dietary fat restriction produces a moderate cholesterol response in E3/E3 individuals
- Dietary fiber, fish oils, and exercise generally improve the lipid profile in this genotype
- Alcohol appears to have a neutral effect on LDL-C
- Avoid smoking, which increases risk of coronary heart disease in this genotype
- E3/E3 individuals generally respond well to statins and would therefore likely respond to statin mimetics such as inositol hexaniacinate, red rice yeast, and policosanol
- Hormone replacement therapy generally improves the lipid profile in all genotypes, including post-menopausal E3 carriers

Key

- \- \- Neither chromosome carries the genetic variation.
- \+ \- One chromosome (of two) carries the genetic variation.
- \+ \+ Both chromosomes carry the genetic variation.

(You inherit one chromosome from each parent)

+ ↑ Gene activity increased
+ ↓ Gene activity decreased

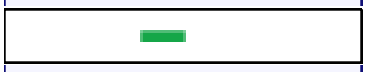
GENOVA DIAGNOSTICS

CYP1B1 — Cytochrome p450 1B1 : DETOXIFICATION

Location:
Chromosome 2

L432V
Your Genotype:

N453S
Your Genotype:



CYP1B1 is a Phase I detoxification enzyme responsible for the 4-hydroxylation of estrogen as well as the activation of environmental toxins such as polycyclic aromatic hydrocarbons, PCBs, and aflatoxin B1.

Health Implications

- Hyper-induction of CYP1B1 upon exposure to its substrates or inducers
- Increased production of 4-hydroxyestrogens and potentially carcinogenic compounds
- Tendency for lower 2:16±-hydroxyestrone ratio (higher risk of breast cancer)
- Increased risk of breast cancer, especially if xenobiotic exposure (e.g., PAHs), high body mass index, estrogen therapy >= 4 yrs, or coexisting CYP1A1 polymorphism (I462V)
- Possible increased risk of cancer of the ovary, uterus, prostate, and lung (esp. if exposed to second-hand smoke)

Treatment Options

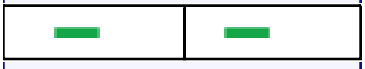
- Minimize exposure to xenobiotics (e.g., PAHs) and xenoestrogens (e.g., organochlorines), which increase CYP1B1 activity
- Maintain a diet rich in antioxidants (colorful fruits and vegetables), consider supplementation
- Consider redirecting estrogen metabolism away from 4-hydroxylation with cruciferous vegetables and/or agents such as indole 3-carbinol (I3C), diindolylmethane (DIM), fish oils, or rosemary
- Caution using long-term estrogen therapy, especially conjugated equine estrogens, which are preferentially 4-hydroxylated. Combined estrogen/progestin therapy produces the greatest breast density in carriers of the SNP
- Carcinogen-induced DNA damage may be minimized by agents such as curcumin, black cohosh, genistein, and DHEA

MTHFR — 5,10-methyltetrahydrofolate reductase : METHYLATION

Location:
Chromosome 1

C677T
Your Genotype:

A1298C
Your Genotype:



5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, facilitating the formation of methyltetrahydrofolate, a required cofactor in the remethylation of homocysteine (Hcy) to methionine.

Health Implications

- Heterozygosity for 677 (-/+) results in 30-40% reduction in MTHFR enzyme activity
- Increased risk of elevated homocysteine, esp. if low levels of B vitamins
- Possible methylation impairment, including disrupted neurotransmitter metabolism and synthesis of DNA, carnitine and coenzyme Q10
- Increased risk of autism, depression, schizophrenia, neural tube defects, cardiovascular disease, essential hypertension, diabetic retinopathy, osteoporosis, and cancers of the stomach
- Low levels of vitamins B2, B6, B12, and/or folate often determines the risk of these associated disorders

Treatment Options

- Ensure adequate intake of folate-rich green vegetables
- Consider supplementation with folic acid (or folinic acid or 5-methyltetrahydrofolate), vitamins B2, B3, B6 (pyridoxal 5-phosphate), B12 (or methylcobalamin), and betaine (trimethylglycine)

COMT — Catechol-O-MethylTransferase : METHYLATION

Location:
Chromosome 22.11q
V158M
Your Genotype:

COMT is a key enzyme in the deactivation of catechol compounds such as catecholamines, estrogens, various chemicals, and toxins. COMT modulates the neurotransmitter functions of dopamine and norepinephrine.

Health Implications:

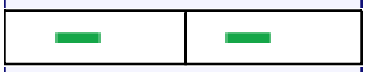
- 3-4-fold reduction in COMT enzyme activity with increased bioavailability of catecholamines and impaired methylation of catechol estrogens
- Increased risk of nervousness, anxiety, or panic disorder
- Increased risk of breast cancer, esp. when coupled with cumulative estrogen exposure
- Reduced pain threshold and increased risk of fibromyalgia
- Increased risk of acute coronary events if also high homocysteine or heavy coffee consumption; increased risk of hypertension, at least among men
- Increased fracture risk, esp. in men; deficient exercise has a greater adverse effect on bone density compared to other genotypes
- In bipolar patients, more rapid switching between depressive to hypomanic episodes

Treatment Options:

- Ensure adequate B6, B12, folate, magnesium, betaine, and methionine to support formation of S-adenosylmethionine and prevent elevated homocysteine; S-adenosylhomocysteine inhibits COMT activity
- Ensure adequate anti-oxidants to prevent oxidation of dopamine and pro-carcinogenic 4-hydroxyestrogens
- Caution using amphetamine-based medications, avoid chronic stress
- Exercise caution using MAO inhibitors, tricyclics, or stimulants including Ritalin®, in bipolar disorder patients
- Inferior anti-depressant response to mirtazapine (Remeron®) or paroxetine (Paxil®)
- Parkinson's patients may respond to lower doses of levodopa and benefit from vitamin B6

TNF-α — Tumor Necrosis Factor-alpha: INFLAMMATION

Location:
Chromosome 6
-308G-A
Your Genotype:



TNF-α is a pro-inflammatory cytokine secreted from activated macrophages that plays an important role in host defense. Excessive TNF-α release can lead to inflammatory reactions and oxidative stress.

Health Implications

- Decreased production of TNF-α, decreased inflammatory tendency and oxidative stress
- Decreased risk of autoimmune disease, osteoporosis, insulin resistance
- May be associated with increased risk of some cancers because of TNF-α's anti-neoplastic properties

Treatment Options

- Risk of inflammatory disorders is minimal
- Diet and lifestyle associated with minimizing cancer risks is prudent

IL-6 — InterLeukin-6 : INFLAMMATION

Location:
Chromosome 7
-174G - C
Your Genotype:

IL-6 is a TH-2 cytokine that promotes maturation of antibody-producing B-cells. IL-6 mediates inflammatory and stress-induced responses.

Health Implications

· *Reduced* IL-6 production and risk of inflammatory responses
· Paradoxical increased risk of elevated C-reactive protein or fibrinogen
· Increased risk of insulin resistance and/or higher body mass index
· Increased risk of Type II diabetes in obese individuals and those with TNFα SNP

Treatment Options

· Reduce any visceral obesity; improve insulin sensitivity
· Minimize intake of refined carbohydrates
· Avoid trans fats, ensure adequate intake of Ω-3 fatty acids

VDR — Vitamin D Receptor : HORMONAL BONE FORMATION

Location:
Chromosome 12
BsmI RFLP
Your Genotype:

VDR is an intracellular hormone receptor that specifically binds the active form of vitamin D and interacts with target-cell nuclei to produce effects.

Health Implications

· Slight impairment of vitamin D receptor with resistance to vitamin D3
· Slightly increased risk of impaired calcium absorption, increased bone loss, lower bone mineral density, and enhanced bone lead accumulation
· Moderately reduced risk of prostate cancer

Treatment Options

· Carriers of the (+) allele benefit from vitamin D supplementation
· Ensure adequate calcium (Ca) intake; studies suggest minimum of 900 mg/day
· Vitamin K may help to compensate for the higher risk of bone loss
· Caffeine intake >300 mg/day may accelerate bone loss, especially when low calcium intake
· Favorable bone response to etidronate and raloxifene and HRT

CYP1A1 — Cytochrome p450 1A1 : DETOXIFICATION

Location:
Chromosome 15

***2A (MSPI)**
Your Genotype:

***2C (I462V)**
Your Genotype:

Cytochrome P450 1A1 (CYP1A1) is a Phase I detoxification enzyme found in extrahepatic tissues such as intestine, lung, skin, lymphocytes and placenta. CYP1A1 primarily metabolizes carcinogens such as polycyclic aromatic hydrocarbons (often activating them to carcinogens) but is also responsible for the 2-hydroxylation of estrogen.

Health Implications

· Baseline "normal" CYP1A1 enzyme activity

· "Normal" degree of procarcinogen activation upon exposures to substrates

Treatment Options

· Regardless of CYP1A1 genotype, it is recommended to minimize exposure to CYP1A1 inducers such as polycyclic aromatic hydrocarbons (e.g. cigarette smoke and well-done meats), heterocyclic amines (e.g., fried meat), PCBs (e.g., contaminated fish or waste), and dioxins (e.g., contaminated meats, fish and dairy, chlorine bleaching, PVC plastics, incineration)

· Maintain a diet rich in antioxidants (colorful fruits and vegetables)

GSTM1 — Glutathione S-Transferase mu-1 : DETOXIFICATION

Location:
Chromosome 1

Your Genotype:

ABSENT

The GSTM1 gene is either PRESENT or ABSENT (also called Null). If either copy is present, it is termed PRESENT. If both copies are absent, it is termed ABSENT.

GST is responsible for Phase II detoxification of xenobiotics, carcinogens, and products of oxidative stress. GSTM1 is located primarily in the liver.

Health Implications

· GSTM1 enzyme activity is absent, with reduced detoxification capacity

· Increased risk of toxic burden, oxidative stress, atopic asthma, lung problems, cancer, chemical sensitivity, and coronary artery disease

· Decreased risk of cancer, only with high intake of cruciferous vegetables

Treatment Options

· Eat cruciferous vegetables and allium foods to reduce cancer risk

· Eat a diet rich in antioxidants (colorful foods), consider supplementation

· Ensure availability of glutathione precursors and cofactors

· Limit glutathione depletion with α-lipoic acid, milk thistle, or taurine

· Minimize exposure to xenobiotics, including PAHs and toxic metals

GSTP1 — Glutathione S-Transferase pi-1 : DETOXIFICATION

Location:
Chromosome 11
A114V
Your Genotype:

—	—

I105V
Your Genotype:

—	—

GST is responsible for Phase II detoxification of xenobiotics, carcinogens, steroids, heavy metals, and products of oxidative stress. GSTP1 is located primarily in the brain and lungs.

Health Implications

· Polymorphisms are associated with either higher or lower enzyme activity, depending on specific environmenta exposures; therefore, the (-/-) genotype may still increase risk for some disorders. The I105V snp is the more significant of the two.

· The I105V genotype (-/-) is associated with slightly increased risk of some cancers (especially if exposed to cigarette smoke), also atopy, xenobiotic-induced asthma, and COPD

Treatment Options

· Ensure availability of glutathione precursors and cofactors, e.g., methionine-rich foods, NAC, L-glutamine, glycine, Mg, B6

· Eat a diet rich in antioxidants (colorful foods), consider supplementation

· Minimize exposure to xenobiotics, including polycyclic aromatic hydrocarbons (e.g., cigarette smoke) and toxic metals

GP3A PL(A) — Platelet Glycoprotein IIIa : COAGULATION

Location:
Chromosome 17
PL(A1)/ PL(A2)
Your Genotype:

—	—
A1	A1

The GP3A polymorphism is a L33P change that results from the substitution of cytosine for thymidine at position 1565. Clinical studies commonly refer to this change as PL(A1) -> PL(A2).

GP3A is a protein component of the platelet fibrinogen receptor IIbIIIa, playing a pivotal role in platelet aggregation and thrombus formation.

Health Implications

· Decreased platelet aggregability and decreased risk of clot formation

· Greater risk of perioperative bleeding due to longer bleeding time

Treatment Options

· Aspirin and oral platelet antagonists are most effective in this genotype

· This genotype may be less sensitive to platelet - inhibiting effects of estrogen

PAI-1 — *Plasminogen Activation Inhibitor-1 : COAGULATION*

Location:
Chromosome 7
Del/Ins (4G/5G)
Your Genotype:

4G	5G
	

The PAI-1 polymorphism represents a single base-pair guanine (hence 5G->4G) in the promoter region. 5G is the norm and 4G is the variant or polymorphism.

PAI-1, present in platelets and vascular endothelium, decreases activation of plasminogen, inhibiting fibrinolytic activity and increasing clots.

Health Implications

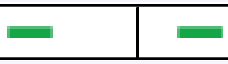
- Higher PAI-1 levels and moderately increased risk of thrombosis
- Possible increased risk of periodontitis, asthma and allergic disease, and PCOS
- Slightly increased risk of obesity, especially in post-menopausal women

Treatment Options

- Evaluate insulin resistance; thiazolidinediones and metformin tend to reduce PAI-1
- PAI-1 is reduced by weight reduction and regular exercise
- Avoid smoking, which increases PAI-1 and risk of restenosis
- Minimize stressors, high intake of saturated fat, and alcohol
- ARBs reduce PAI-1 levels and ACE inhibitors are particularly effective in hypertensive patients with genotype
- Hormone therapy and DHEA supplementation reduces PAI-1, decreasing clots post-menopausally
- Nattokinase dissolves fibrin and inactivates PAI-1

FACTOR II — *Factor II (Prothrombin) : COAGULATION*

Location:
Chromosome 11
G20210A
Your Genotype:



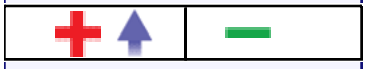
Factor II is also known as prothrombin, which is converted to its active form, thrombin, and forms the essential part of a blood clot.

Health Implications

- Normal levels of prothrombin
- No increased risk of venous thromboembolism

Treatment Options

- None indicated

FACTOR V	*Factor V (Leiden) : COAGULATION*
Location: Chromosome 1 **R506Q** **Your Genotype:** 	Factor V combines with Factor X to convert prothrombin to thrombin, the essential part of a blood clot. Factor Va is held in check by Protein C. **Health Implications** · Elevated levels of thrombin; 7-fold increased risk of clot formation · Increased chance of heart attack and atherosclerosis · Increased risk of miscarriage, pre-eclampsia, and placental abruption **Treatment Options** · Avoid oral contraceptives; risk of DVT increases 35-fold · Avoid oral HRT, smoking, high homocysteine · Platelet activation inhibitors include: fish oils, garlic, onions, ginger, ginkgo biloba, thyme, rosemary, genistein, and aspirin · Glycyrrhizin (licorice) inhibits conversion of prothrombin to thrombin · Exercise caution with hypertension

This test has been developed and its performance characteristics determined by Genova Diagnostics, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration.

Commentary is provided to the practitioner for educational purposes, and should not be interpreted as diagnostic or treatment recommendations. Diagnosis and treatment decisions are the responsibility of the practitioner.

The accuracy of genetic testing is not 100%. Results of genetic tests should be taken in the context of clinical representation and familial risk. The prevalence and significance of some allelic variations may be population specific.

Any positive findings in your patient's test indicate genetic predisposition that could affect physiologic function and risk of disease. We do not measure every possible genetic variation. Your patient may have additional risk that is not measured by this test. Negative findings do not imply that your patient is risk-free.

DNA sequencing is used to detect polymorphisms in the patient's DNA sample. The sensitivity and specificity of this assay is <100%.

CHECKLIST (PRIOR TO SHIPPING)

1. Cotton Swabs

❑ Swabs are returned to the original Cotton Swab Package

❑ Cotton Swabs Package is sealed in the Letter Envelope

2. Specimen Collection Label

❑ Label is filled out and adhered to the Letter Envelope

3. Test Requisition Form with Payment

❑ Test Requisition Form is complete - Test is marked, Patient's first and last name, date of birth, gender, date of collection

❑ Test requistion is placed in the collection kit envelope.

❑ Payment is included

GENOVA DIAGNOSTICS

ASHEVILLE • ATLANTA • LONDON

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GENOVATIONS®

This specimen collection kit can be used for the following tests:

CardioGenomicPlus™ Profile *

DetoxiGenomic® Profile *

EstroGenomic™ Profile *

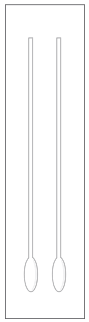
Sub Panel Estrogen Metabolism *

Sub Panel Hypercoagulation *

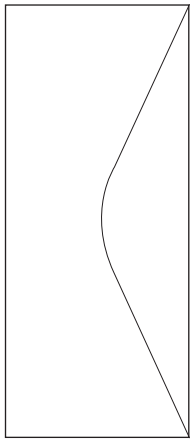
ImmunoGenomic® Profile *

NeuroGenomic™ Profile *

* Not Available in New York



Cotton Swabs and Package



Letter Envelope

SPECIMEN

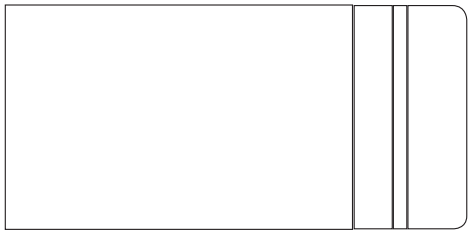
Buccal swab

COLLECTION MATERIALS

- 2 Cotton Swabs
- Returnable Cotton Swabs Package
- Letter Envelope

SHIPPING MATERIALS*

- Collection Kit Package
- Test Requisition Form
- FedEx® Billable Stamp
- Specimen Collection Label



Collection Kit Package

International shipping may vary, please see shipping instructions for more details.

IMPORTANT:

All patient specimens require two unique identifiers (***patient's name and date of birth***), as well as ***date of collection***. **Patient's first and last name, date of birth, gender,** and **date of collection** must be recorded on the **Test Requisition Form** as well as all tube(s) and/or cup(s), using a permanent marker, or the test may not be processed.

GENOVA DIAGNOSTICS

Please read all instructions carefully before beginning.

PATIENT PREPARATION

- Specimen **must be collected immediately** upon rising. **Do not practice** normal oral hygiene routine, **do not eat or drink.**
- **Prior to collection:** The night before collection, use your normal nightly routine of brushing and flossing of teeth, but **do not use mouthwash.**
- **Morning of Collection:** On the morning of collection, do not eat, brush or floss your teeth, use mouthwash, chew gum or use any tobacco, or coffee products. You may drink ONLY water before specimen collection. Just prior to collection, wash hands completely with hand soap.

SALIVA COLLECTION

1. **Write** patient's **first and last name, date of birth**, **gender** and **date of collection** on the Test Requisition Form.
   IMPORTANT: To ensure accurate test results you **MUST** provide the requested information.
2. **Peel** open the package labeled, "Sterile Cotton Tipped Applicator." *Only peel back the package far enough to remove the cotton swab applicator*. Keep the packet intact. (See Figure 1).
3. **Remove** one applicator taking care to avoid contact with the cotton tip.
4. **Open** your mouth widely and insert applicator. For at least 30 seconds, **aggressively scrape** the inside of your cheek using a back and forth, and up and down motion. Be sure to **rotate** the applicator several times to ensure the swab collects a sufficient amount of cheek cells. In addition, **swab** between the cheek and gums. (See Figure 2)
   Note: If there is not enough DNA collected on the applicator, a recollection will be required.
5. **Remove** the applicator from your mouth and allow cotton tips to air dry for 15-20 minutes (See Figure 3A) before placing it back into the original packaging, cotton swab first. (See Figure 3B)
6. **Repeat** the collection process (steps 1-3) with the second applicator on your opposite cheek.

SPECIMEN PREPARATION

1. **Place** the package containing the two collected specimen swabs into the letter envelope. Seal the letter envelope.
2. **Print name and collection date** on specimen collection label. **Place** the specimen collection label on the letter envelope.
3. **Seal** and **place** the letter envelope into the collection kit envelope.
4. **Fill** out the Test Requisition by completing all patient and billing information, including the date of collection. **Sign** the form and **place** it back inside the collection kit package.
5. **Place** FedEx billable stamp on the collection kit package and **call** 1-800-GoFedEx to schedule a pick up.

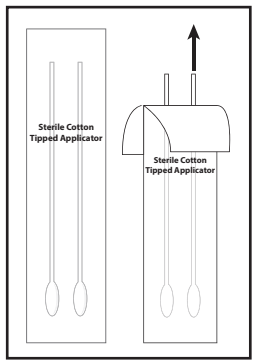


figure 1

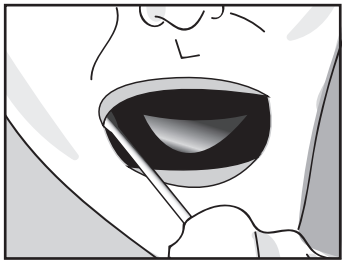


figure 2

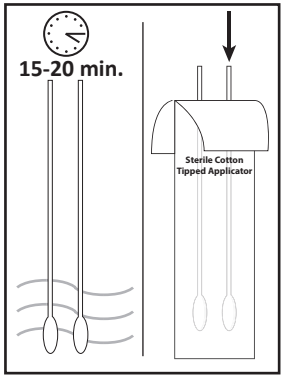
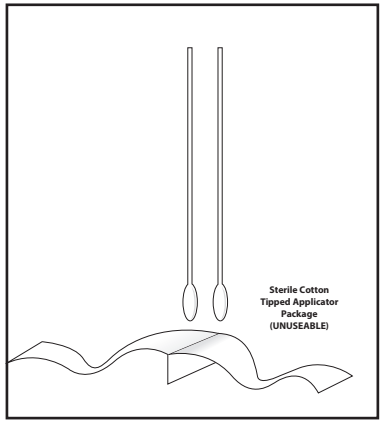


figure 3A figure 3B

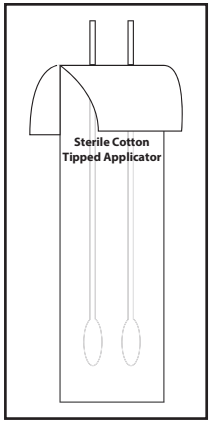
(REPEAT FIGURES 1 - 3)

NO



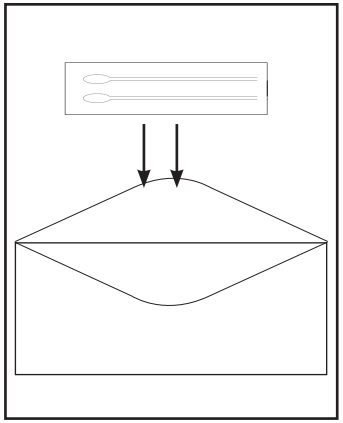
Package NOT intact
SPECIMEN UNUSABLE!!

YES



Package Intact
Specimen Usable

YES


Insert Specimens into Letter Envelope