Thrombophilia: A Case Study

Institute of Medicine
Roundtable on Translating Genomic-Based Research for Health
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Slides marked with * were taken from an excellent slide presentation by:

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Nichols Institute

Thrombophilia: Failure of the Inherent Anticoagulation Defense System
Thrombophilia

- 2 million Americans affected with DVT each year
- 600,000 of these develop pulmonary embolism (PE)
- 60,000 of those with DVT and PE die each year

DVT, deep vein thrombosis.
Thrombophilia

- 5% - 8% of population affected by genetic defects leading to thrombosis predisposition
- 25% suffer chronic swelling, skin ulceration, and impaired mobility secondary to “venous hypertension”
DVTs

- 50% of “unprovoked” DVT cases associated with hereditary thrombophilia
- 60% of DVT cases in pregnant women associated with factor V Leiden mutation (A1698G)
- DVT often associated with multiple genetic and acquired risk factors
- DVT responds to prolonged anticoagulant therapy (e.g., LMWH, coumarin-based ACs)

Pulmonary embolism

- 4 million patients present to U.S. emergency departments with shortness of breath each year

- Shortness of breath = heart failure or pulmonary embolism (PE)

- 60% of patients who die in the hospital have PE

- PE diagnosis missed in 70% of hospital cases

- 10% of patients with acute PE die within first 60 minutes

Clagett GP. Chest. 1998;114(Suppl 5):531S-560S.
Clinical DVT Risk Factors

- Age >60 y
- Extensive surgery*
- Marked immobility, pre- or postoperative
- Major orthopedic surgery (eg, hip, knee)
- Fracture of pelvis, femur, or tibia
- Surgery for malignant disease
- Postoperative sepsis
- Major medical illness
  - Heart failure
  - Inflammatory bowel disease
  - Sepsis
  - Myocardial infarction
  - Stroke

*Risk of postoperative thrombosis increases as patient’s age and surgery duration increases; risk also increases with presence of varicose veins and obesity.
Thrombophilia is often multigenic

Multiple risk factors raise the risk of thrombosis
Frequency of Biological Risk Factors

- Factor VIII excess (30%)
- Activated protein C resistance (APCR), Factor V mutation (20%)
- Prothrombin (Factor II) mutation (15%)
- Hyperhomocysteinemia, MTHFR mutation (10%)
- Protein C (10%)
- Protein S (10%)
- Antithrombin (4%)
- Unknown (5%)
- LA (3%)
- Other (<1%)
- Activated protein C resistance (APCR), Factor V mutation (20%)
Risk of Recurrent Venous Thromboembolism

<table>
<thead>
<tr>
<th>Thrombophilic Defect</th>
<th>Estimated Relative Risk</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>De Stefano V. Br J Haematol. 2001;113:630-635.</td>
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<td></td>
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<td>Rance A. Thromb Haemost. 1997;77:221-222.</td>
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</tbody>
</table>

* pooled using the using the Mantel-Haenszel method

Who and When to Test

• In 2001 and 2005, the American College of Medical Genetics (ACMG) issued guidelines for \textit{F5} and \textit{F2} testing, indicating that testing in certain ethnic groups may have utility in the following circumstances\[i]\,[iii]
  – Age < 50, any venous thrombosis.
  – Venous thrombosis in unusual sites (such as portal hepatic, mesenteric, and cerebral veins).
  – Recurrent venous thrombosis.
  – Venous thrombosis and a strong family history of thrombotic disease.
  – Venous thrombosis in pregnant women or women taking oral contraceptives.
  – Myocardial infarction in female smokers under age 50.

• Testing may also be considered in the following situations:
  – Venous thrombosis, age >50, except when active malignancy is present.
  – Relatives of individuals known to have Factor V Leiden
  – Woman with recurrent pregnancy loss or unexplained severe preeclampsia, placental abruption, intrauterine fetal growth retardation, or stillbirth.

What to Test

• The **Verigene F5 Nucleic Acid Test** is an *in vitro* diagnostic for the detection and genotyping of a single point mutation (G to A at position 1691; also known as Factor V Leiden) of the human Factor V gene (*F5*; Coagulation Factor V gene) in patients with suspected thrombophilia, from isolated genomic DNA. The test is intended to be used on the Verigene System.

• The **Verigene F2 Nucleic Acid Test** is an *in vitro* diagnostic for the detection and genotyping of a single point mutation (G to A at position 20210) of the human Factor II gene (*F2*; prothrombin gene) in patients with suspected thrombophilia, from isolated genomic DNA. The test is intended to be used on the Verigene System.

• The **Verigene MTHFR Nucleic Acid Test** is an *in vitro* diagnostic for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5,10 methylenetetrahydrofolate reductase gene (*MTHFR*) in patients with suspected thrombophilia, from isolated genomic DNA. The test is intended to be used on the Verigene System.
Prevalence of the FV 1691G>A mutation in various ethnic groups

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Heterozygotes</th>
<th>Homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>3-8%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Hispanic Am</td>
<td>2.2%</td>
<td>Very Rare</td>
</tr>
<tr>
<td>Native Am</td>
<td>1.25%</td>
<td>Very Rare</td>
</tr>
<tr>
<td>African Am</td>
<td>1.23%</td>
<td>Very Rare</td>
</tr>
<tr>
<td>Asian Am</td>
<td>1.23%</td>
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</table>
Prevalence of the F2 20210G>A mutation in various ethnic groups

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<tr>
<th>Ethnic Group</th>
<th>Heterozygotes</th>
<th>Homozygotes</th>
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</thead>
<tbody>
<tr>
<td>Southern Eur</td>
<td>3.0%</td>
<td>Very Rare</td>
</tr>
<tr>
<td>Northern Eur</td>
<td>1.7%</td>
<td>Very Rare</td>
</tr>
<tr>
<td>Hispanic Am</td>
<td>Very Rare</td>
<td>Very Rare</td>
</tr>
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The \textit{MTHFR} 677C>T mutation has been observed in all ethnic groups and varies widely in prevalence, from low levels (~6.6\%) in Africa to high levels (~44.9\%) in certain populations of South America.

In Recommendation 4 of the ACMG guideline on Factor V Leiden mutation testing, it is noted that hyperhomocysteinemia is considered a potential risk factor for thrombophilia. Patients with classic homocystinuria are at extremely elevated risk of thromboembolism. Since \textit{MTHFR} mutations can lead to elevated levels of plasma homocysteine and subsequent higher risk of thrombosis, laboratorians and physicians have requested.
How to Test

- PCR-based systems (target amplification)

- Non-PCR-based systems (no target amplification)

- FDA premarket notification (510(k)) or analyte-specific reagent (ASR)
How to Test
(FDA-cleared tests only)

Factor II (prothrombin)*
- **AutoGenomics, Inc.:** Carlsbad, CA - INFINITI™ System Assay for Factor II - PCR and Detection Primer Extension - 510(k)
- **Roche Diagnostics:** Pleasanton, CA - Factor II (prothrombin) G20210A kit - Real-Time PCR - 510(k)
- **Nanosphere, Inc.:** Northbrook, IL - Verigene® F2 Nucleic Acid Test - Multiplex Gold Nanoparticle Probes - 510(k)

Factor V Leiden*
- **AutoGenomics, Inc.:** Carlsbad, CA - INFINITI™ System Assay for Factor V - PCR and Detection Primer Extension - 510(k)
- **Roche Diagnostics:** Pleasanton, CA - Factor V Leiden kit - Real-Time PCR - 510(k)
- **Nanosphere, Inc.:** Northbrook, IL - Verigene® F5 Nucleic Acid Test - Multiplex Gold Nanoparticle Probes - 510(k)

Factor II (Prothrombin) and Factor V Leiden*
- **AutoGenomics, Inc.:** Carlsbad, CA - INFINITI™ System - Assay for Factor II & Factor V - PCR and Detection Primer Extension - 510(k)
- **Nanosphere, Inc.:** Northbrook, IL - Verigene® F5/F2 Nucleic Acid Test - Multiplex Gold Nanoparticle Probes - 510(k)

Factor II (Prothrombin), Factor V Leiden and MTHFR
- **Nanosphere, Inc.:** Northbrook, IL - Verigene® F5/F2/MTHFR Nucleic Acid Test - Multiplex Gold Nanoparticle Probes - 510(k)

* Third Wave Technologies – Madison, WI – no PCR – 510(k)
Prothrombin (Factor II) 20210G→A Mutation

A, adenine; G, guanine.
Synergistic Effects of Thrombotic Risk Factors

<table>
<thead>
<tr>
<th>Factor V Leiden</th>
<th>Factor II$^a$</th>
<th>Thrombotic Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>1.0</td>
</tr>
<tr>
<td>Abnormal</td>
<td>Normal</td>
<td>2.8</td>
</tr>
<tr>
<td>Normal</td>
<td>Abnormal</td>
<td>2.4</td>
</tr>
<tr>
<td>Abnormal</td>
<td>Abnormal</td>
<td>22.8</td>
</tr>
</tbody>
</table>

$^a$Prothrombin 20210G→A mutation.
## Synergistic Effects of Thrombotic Risk Factors

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<th>Thrombotic Risk</th>
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<td>Normal</td>
<td>2.8</td>
</tr>
<tr>
<td>Normal</td>
<td>Abnormal</td>
<td>1.8</td>
</tr>
<tr>
<td>Abnormal</td>
<td>Abnormal</td>
<td>16.5</td>
</tr>
</tbody>
</table>
## Synergistic Effects of Thrombotic Risk Factors

<table>
<thead>
<tr>
<th>Factor V Leiden</th>
<th>Factor II(^a)</th>
<th>MTHFR</th>
<th>Thrombotic Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
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<td>1.0</td>
</tr>
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<td>Normal</td>
<td>2.8</td>
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<tr>
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<td>Abnormal</td>
<td>1.8</td>
</tr>
<tr>
<td>Normal</td>
<td>Abnormal</td>
<td>Normal</td>
<td>2.4</td>
</tr>
<tr>
<td>Abnormal</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>56.5</td>
</tr>
</tbody>
</table>

\(^a\)Prothrombin 20210G→A mutation.
Case Study #1

- 61 year-old white male with multiple myeloma (in remission)
- Receiving no treatment
- Flies to Singapore for vacation
- Experiences discomfort in right leg, no swelling, no rubor
- 2 days later, sudden dyspnea, left pleuritic chest pain
- Hospitalized with DVT/PE
- Returns home 2 weeks later on coumadin
Case Study #1: Laboratory Workup

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>12.4 g/dL</td>
</tr>
<tr>
<td>Platelet count</td>
<td>177,000/μL</td>
</tr>
<tr>
<td>WBC</td>
<td>3,100/μL</td>
</tr>
<tr>
<td>INR</td>
<td>2.2</td>
</tr>
<tr>
<td>D-dimer</td>
<td>&lt;0.79 μg/mL FEU</td>
</tr>
<tr>
<td>APCR</td>
<td>1.3 ratio</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>87%</td>
</tr>
<tr>
<td>Protein C</td>
<td>&lt;41%</td>
</tr>
<tr>
<td>Protein S</td>
<td>22%</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>Homozygous</td>
</tr>
<tr>
<td>Follow-up duplex ultrasound</td>
<td>Residual thrombus in iliac system and IVC</td>
</tr>
</tbody>
</table>
Case Study #2

• 39 year-old, physically active, white male bicyclist develops rapidly progressive drawing pain in right calf after 40-mile event

• History of DVT in left lower extremity after laparoscopic knee surgery at age 21

• Mother on coumadin

• Father died mysteriously of unknown causes at age 51 years

• Sister has had recurrent miscarriages
<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PT</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.4 sec. (normal, 10 – 12)</td>
</tr>
<tr>
<td>aPTT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.0 sec. (normal, &lt;36 sec.)</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>R506Q heterozygous</td>
</tr>
<tr>
<td>Prothrombin gene</td>
<td>G20210A heterozygous</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Lupus anticoagulant screen</td>
<td>Negative</td>
</tr>
</tbody>
</table>

MRI, vena cava: Thrombus to vena cava; duplicate cava; old thrombosis noted

<sup>a</sup>Tested prior to initiation of anticoagulant therapy.
<sup>b</sup>Tested after initiation of coumadin therapy; INR 3.1.
Conclusion

Long-term anticoagulation indicated

**Cost-Effective Hypercoagulopathy Workup**

**Below Age 45**

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCR</td>
</tr>
<tr>
<td>Protein C</td>
</tr>
<tr>
<td>Protein S</td>
</tr>
<tr>
<td>Antithrombin</td>
</tr>
<tr>
<td>Prothrombin mutation</td>
</tr>
<tr>
<td>Homocysteine</td>
</tr>
<tr>
<td>LA (aPTT) and ACA/APS</td>
</tr>
</tbody>
</table>

If APCR is abnormal or borderline normal, perform factor V Leiden mutation analysis.

If aPTT is abnormal, complete LA workup.

If homocysteine is abnormal, perform MTHFR mutation analysis.
Cost-Effective Hypercoagulation Workup

Over Age 45

- APCR
- Homocysteine
- Prothrombin mutation
- LA (aPTT) and ACA/APS

If APCR is abnormal or borderline normal, perform factor V Leiden mutation analysis.

If aPTT is abnormal, complete LA workup.

If homocysteine is abnormal, perform MTHFR mutation analysis.
Verigene® System

- Pipette genomic DNA into fluidics cartridge
- Disposable, single-use, self-contained cartridge (All reagents on board)
- Control station and user interface
- Image analysis and results generation
- Performs entire assay
- Random access
Strategy for Higher Density of Multiplexing

Amplification Ag Shell

gDNA

Au Nanoprobe

T

A

C

MT

WT

Direct Nanoprobe Format

Mediator Probe

Mediator Format [High Multiplexing Format]
Multiplex SNP Genotyping in the Human Genome

Discrimination Factors (DF)
Average Value +/- Stdev

\[
DF = \frac{(S_{\text{wt}} - S_{\text{mut}})}{(S_{\text{wt}} + S_{\text{mut}})}
\]
Discussion

How did the manufacturer decide what variants would be measured and how results were to be interpreted?
- Combination of published data, algorithms, guidelines, and past FDA submissions
- One of the most common genetic tests performed routinely
- Value proposition is the multiplexing of Factor V and Factor II together ubiquitously (any type of lab - no risk of contamination), with minimal lab technologist expertise (unlike PCR)
- Good proof of concept assay due to its broad use

What was the business model for the test?
- Our features and benefits directed us to pharmacogenetic testing
- Proof of concept and anchor assay for most hospitals who perform or looking to bring on molecular testing

What obstacles have the manufacturers or distributors experienced? Are other obstacles foreseen?
- Market development, continuous data accrual, reimbursement, and education
- Keeping up with demand

What regulatory issues had to be addressed in bringing the test to market?
- Pre-IDE process
- Standard 510(k) process
- Reproducibility studies
Discussion

What evidence base was needed at different stages of translation? What were the barriers or facilitators to developing the needed evidence?

- Basically, evidence that we could match PCR in terms of accuracy and could multiplex all 3 variants in 90 minutes without PhDs running the test.

Are there evidence-based practice guidelines? Are guidelines from different sources consistent?

- From the laboratory and from the ACMG and AMP which are consistent.

Does the test require decision support tools or confirmatory testing?

- Limited to none, only clinical-based tools that define what each clinician should do with the information.

Has post-market research been performed? Are there data on outcomes of testing in clinical settings? Has use of test changed with experience?

- Collecting currently.

What is the current level of test use? Who is using the test?

- Just launched in October so still early.
- Acceptance high – 30-40 institutions and growing in all types of hospitals including medium-sized hospitals that are just starting molecular programs.
discussion

are there any data on perceived value of testing on the part of consumers or providers?
- data being gathered
- acceptance level is a good sign

is the test reimbursed by payers?
- Some are paying and some want more data
- Currently using individual step codes since no specific test codes exist

What lessons can we learn?
- Need broader menu with the right test mix – knew this all along (e.g., hypercoagulation panel, warfarin metabolism panel, cystic fibrosis panel)
- Warfarin sensitivity test with FV/FII/MTHFR is an excellent combination
- Can run high-sensitivity protein tests (e.g., cTnI, PSA) on same platform, side-by-side concurrently