Genes and Outcomes in Sepsis

Keith R. Walley, MD
St. Paul’s Hospital
University of British Columbia
Vancouver, Canada

Conflict: Sirius Genomics Inc.
# Role of Heredity in Sepsis\(^{(1)}\)

<table>
<thead>
<tr>
<th>Parents (Death of a biological parent &lt;50 years)</th>
<th>Relative Risk of Adoptee Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>1.2</td>
</tr>
<tr>
<td>Cardiovascular Disease</td>
<td>4.5</td>
</tr>
<tr>
<td>Infectious Disease</td>
<td>5.8</td>
</tr>
</tbody>
</table>

1. Sorensen TI et al. NEJM 1988; 318: 727
Genetic Polymorphisms
After Dr. Jean-Daniel Chiche

• A change in code can alter outcome

- Hot message - good news
- Hot mess - a big problem
- Hot massage - something else
Single Nucleotide Polymorphisms

• Most common genetic variant
• Common SNPs (minor allele frequency > 5%) occur every ~500 base pairs
• All listed on HapMap website
• Can genotype 1 million SNPs for ~$500
UCSC Genome Browser on Human (GRCh37/hg19) Assembly
Severe Sepsis / Septic Shock

- 20 - 40% mortality
- Antibiotics, volume, adrenergic agonists, vasopressin, steroids

**HYPOTHESIS** – polymorphisms in genes relevant to therapy alter response to therapy.
1. \( \beta_2 \)- adrenergic receptor
2. Response to vasopressin
β2- Adrenergic Receptor

Asthma
Cys/Gly/Gln homozygotes are hypo-responders to salbutamol

Cys/Gly/Gln marked by A allele of ADRB2 rs1042717 G/A polymorphism

Higher heart rate

Greater norepinephrine dose (hypo-responders)
AA genotype associated with IL-6 production by cells
Good response to steroids

No Acute Steroids

Acute Steroids

Survival

Days

SPH

VASST

Survival

Days

SPH

VASST

No Acute Steroids

Acute Steroids

SPH

VASST

Survival

Days

SPH

VASST

Survival
β2- Adrenergic Receptor

- Cys/Gly/Gln homozygotes (rs1042717 AA) are hypo-responders to adrenergic agonists and have high mortality.

- Maybe non-adrenergic vasopressors should be considered.

- Steroids may help.
Vasopressin and Septic Shock Trial (VASST)
Kaplan-Meier survival curve
All patients

Log-rank statistic
p = 0.27 day 28
p = 0.10 day 90

Days since initiation of study drug
Probability of survival

Vasopressin
Norepinephrine

0 10 30 50 70 90
0 0.2 0.4 0.6 0.8 1

Days since initiation of study drug
Probability of survival

Vasopressin
Norepinephrine
Low severity of shock stratum
$5 \mu g/min < NE < 15 \mu g/min$

Log-rank statistic
$p = 0.05$ day 28
$p = 0.03$ day 90
Vasopressin V1a receptor genetic variation in voles

“In addition, males overexpressing the V1aR in the ventral pallidal region, but not control males, formed strong partner preferences after an overnight cohabitation, without mating, with a female.”

Also in Humans

Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans.
Walum H et al. PNAS. 105:14153-6, 2008

The dopamine transporter gene (DAT1) polymorphism is associated with premature ejaculation.
Risk of Death Analysis
SPH Severe Sepsis Cohort: Vasopressinase rs18059

28-day mortality (%)

<table>
<thead>
<tr>
<th></th>
<th>TT/CT</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-day mortality (%)</td>
<td>285</td>
<td>81</td>
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</tbody>
</table>

p < 0.09
Identification of 230 SNPs in a huge haplotype block (160 kb) covering the *LNPEP* region and genotyping

1. Identification of 230 SNPs by re-sequencing
2. Genotyping 230 SNPs in the derivation cohort
3. Screening 230 SNPs on 28-day mortality
LNPEP – Vasopressinase SNPs

rs4869317

$P = 4.4 \times 10^{-4}$

Major allele model

(TT vs. AT+AA)

28-day mortality (%)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TT</th>
<th>AT</th>
<th>AA</th>
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<tr>
<td>n.</td>
<td>337</td>
<td>197</td>
<td>55</td>
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</tbody>
</table>
LNPEP – Vasopressinase SNP rs4869317 in Septic Shock

SPH Derivation Cohort

\[ P = 0.0013 \]

Number at Risk

- **AA/AT**: 252, 208, 182, 175, 165
- **TT**: 337, 244, 205, 184, 165

Days

Probability of Survival

VASST Replication Cohort

\[ P = 0.026 \]

Number at Risk

- **AA/AT**: 296, 254, 229, 220, 210
- **TT**: 316, 250, 224, 207, 199

Days

Probability of Survival
LNPEP – Vasopressinase SNP rs4869317 in Septic Shock

Constant infusion during 72 hours
One-compartment model ($C_P = R \frac{1-e^{-kt}}{V_D\kappa}$)

Circulating Vasopressin Levels

Vasopressin Clearance

$P=0.028$

n=21  n=24
**LNPEP rs4869317** accounted for 80% of the variance of peri-operative serum sodium levels in cardiac surgical patients.

**Locus specific heritability**

\[
\frac{\text{Genetic Variance}}{\text{Total Variance}} = 0.800
\]

![Graph showing serum sodium levels for AA/AT and TT genotypes with P=0.045](graph.png)
Vasopressinase
LNPEP rs18059

SPH

VASST

<table>
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<tr>
<th>Genotype</th>
<th>SPH NE n = 81</th>
<th>AVP n = 73</th>
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</thead>
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<tr>
<td>TT/CT</td>
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<tr>
<td>CC</td>
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</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>VASST NE n = 139</th>
<th>AVP n = 140</th>
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</thead>
<tbody>
<tr>
<td>TT/CT</td>
<td></td>
<td></td>
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<tr>
<td>CC</td>
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Vasopressinase (LNPEP)

- LNPEP genotype impacts mortality and response to vasopressin.
- Maybe vasopressin use should be guided by genotype measurement.
Summary

HYPOTHESIS – polymorphisms in genes relevant to therapy alter response to therapy.

1. β2- adrenergic receptor
2. Vasopressinase

• Following confirmatory large prospective studies, gene polymorphisms may help decide who should receive specific therapies when septic.
Co-investigator
Jim Russell

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Funding
Heart & Stroke Foundation
CIHR
Michael Smith Foundation
Sirius Genomics Inc.

Centre for
Heart Lung Innovation
UBC and St. Paul’s Hospital
Plasma PAI-1 / Protein C levels in ARDS

Biological Plausibility: Protein C levels

PROC rs2069912: PROWESS APACHE II ≥ 25 (Mean ± SE)

Placebo

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Plasma Protein C</th>
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<tr>
<td>0</td>
<td>0.4</td>
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<tr>
<td>10</td>
<td>0.6</td>
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<tr>
<td>20</td>
<td>0.8</td>
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<tr>
<td>30</td>
<td>1.0</td>
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</table>

rhAPC

<table>
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<th>Time (days)</th>
<th>Plasma Protein C</th>
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<tbody>
<tr>
<td>0</td>
<td>0.4</td>
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<tr>
<td>10</td>
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<td>20</td>
<td>0.8</td>
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<tr>
<td>30</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Biological Plausibility: PAI-1 levels

PAI-1 rs7242: PROWESS APACHE II ≥ 25 (Mean ± SE, imputed data)

Placebo

rhAPC

[PAI-1] (AU/ml)

Time (days)

GG n=12
GT/TT n=50

GG n=11
GT/TT n=60
Protein C / PAI-1 Combination Genotype

+/+ genotype is defined as:

At least one copy of the responsive C allele of PROC rs2069912 T/C
AND
At least one copy of the responsive T allele of PAI-1 rs7242 T/G
<table>
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<th>Hypothesis Generation</th>
<th>Hypothesis Testing</th>
<th>Clinical Practice Confirmation</th>
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<tr>
<td>St Paul’s Hospital</td>
<td>PROWESS</td>
<td>VASST</td>
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<tr>
<td>(Efficiency)</td>
<td>(Efficacy)</td>
<td>(Efficiency)</td>
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<tr>
<td>Severe sepsis</td>
<td>Severe sepsis</td>
<td>Severe sepsis</td>
</tr>
<tr>
<td>N = 1024</td>
<td>N = 1568</td>
<td>N = 423</td>
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<tr>
<td>High risk of death</td>
<td>High risk of death</td>
<td>High risk of death</td>
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<tr>
<td>Xigris vs. control</td>
<td>Xigris vs. placebo</td>
<td>Xigris vs. control</td>
</tr>
<tr>
<td>N = 49 vs. N = 243</td>
<td>N = 382 vs. N = 370</td>
<td>N = 88 vs. N = 333</td>
</tr>
</tbody>
</table>
Protein C / PAI-1 combination genotype

Absolute Risk Reduction in 28-day mortality due to rhAPC

**SPH**

- **+/+**
- **+/-**
- **-/—**

**PROWESS**

- **+/+**
- **+/-**
- **-/—**

**VASST**

- **+/+**
- **+/-**
- **-/—**

<table>
<thead>
<tr>
<th>Condition</th>
<th>SPH</th>
<th>PROWESS</th>
<th>VASST</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>0.062</td>
<td>&lt;0.0002</td>
<td>0.040</td>
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</table>

IRP+/+ effect (Survival ~ Age + APACHEII + Caucasian + rhAPC)

Meta-analysis p<0.0001
Safety: Serious Adverse Events (SAEs) by combination genotype in PROWESS (APACHE II ≥ 25)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Placebo</th>
<th>Xigris</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRP +/+</td>
<td>25/138</td>
<td>14/124</td>
</tr>
<tr>
<td>IRP +/-</td>
<td>23/178</td>
<td>29/204</td>
</tr>
<tr>
<td>IRP -/-</td>
<td>1/38</td>
<td>7/33</td>
</tr>
</tbody>
</table>

Between genotypes:

- Base: p=0.16
- IRP -/-: p=0.01
Non-Bleeding Serious Adverse Events
All SAEs minus Bleeding SAEs  (APACHE II ≥ 25)

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<tr>
<th>Genotype</th>
<th>Placebo</th>
<th>Xigris</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
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<td>0.006</td>
</tr>
<tr>
<td>IRP +/-</td>
<td>20/178</td>
<td>23/204</td>
<td>0.15</td>
</tr>
<tr>
<td>IRP -/-</td>
<td>1/38</td>
<td>6/33</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Within genotype
- IRP +/+: p=0.006
- IRP +/-: p=0.15
- IRP -/-: p=0.04

Between genotypes
- Base: p=0.022
- Placebo: p=0.005
Protein C SNPs chosen for functional testing

http://uswest.ensembl.org

rs1799808 C/T
-1654
rs1799809 A/G
-1641
rs1158867 C/T
rs2069910 C/T
rs2069913 C/G
rs2069916 C/T

Promoter region

rs1799810 A/T
rs2069912 C/T
rs2069914 G/A
rs2069915 G/A
Binding to rs2069915[G/A] is allele specific

- **EMSA repeated 3 times**
- **Cold competition**
  - Unlabelled probes
  - 50x Molar excess

Labelled probes: G A G A G A

Specific probe: G A G A G A

Scrambled probe: G A G A G A

Nuclear lysate from healthy human liver tissue
Summary of EMSA Assays Measured by Densitometry

<table>
<thead>
<tr>
<th>Average Density (arbitrary units)</th>
<th>Promoter haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1799808 ― rs1799809</td>
</tr>
<tr>
<td></td>
<td>-1654[C/T] ― -1641[A/G]</td>
</tr>
</tbody>
</table>

** t-test, p<0.0003, n = 6
* t-test, p<0.005, n = 3
Summary

HYPOTHESIS – polymorphisms in genes relevant to therapy alter response to therapy.

1. $\beta_2$- adrenergic receptor
2. Vasopressinase
3. Activated Protein C

• Following confirmatory large prospective studies, gene polymorphisms may help decide who should receive specific therapies when septic.
Protein C -1641 in Sepsis

Treatment Response: Protein C
Derivation SPH Severe Sepsis Cohort: rs2069912

28-day mortality (%)

CC/CT
p = 0.1

TT
p = 0.7

Control
N = 224

rhAPC-treated
N = 44

APACHE ≥ 25, Plts > 30,000,
INR <3, Bilirubin <20 µmol/l
Treatment Response: PAI-1
Derivation SPH Severe Sepsis Cohort: rs7242

28-day mortality (%)

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>GT/TT</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>rhAPC-treated</td>
<td>30</td>
<td>40</td>
</tr>
</tbody>
</table>

Control  N = 170
rhAPC-treated  N = 44
Plasma PAI-1 / Protein C levels in ARDS

Ware et al, CCM 2007
Serious Adverse Events (SAE) by Genotype in PROWESS

- IRP +/+ (P = 0.17)
  - Placebo: 25/138
  - Xigris: 14/124

- IRP +/- (P = 0.83)
  - Placebo: 23/178
  - Xigris: 29/204

- IRP +/- (P = 0.021)
  - Placebo: 1/38
  - Xigris: 7/33

Interaction: Base
  - P = 0.16

Interaction: P = 0.01
Summary

HYPOTHESIS – polymorphisms in genes relevant to therapy alter response to therapy.

1. $\beta_2$- adrenergic receptor
2. Protein C / PAI-1

- Following confirmatory large prospective studies, gene polymorphisms may help decide who should receive specific therapies when septic.
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Protein C and PAI-1 combined genotype

Absolute Risk Reduction in 28-day mortality due to rhAPC

SPH
PROWESS
VASST

+/+ has responsive allele for Protein C rs2069912
• High PAI-1 is bad

• Low Protein C is bad

• High PAI-1 / Protein C ratio is bad
Serious Adverse Events (SAE) by IRP Genotype in PROWESS

- IRP ++ (P = 0.17): 25/138
- IRP +- (P = 0.83): 23/178
- IRP -- (P = 0.021): 1/33

Interaction Base

P = 0.01
PAI-1/Protein C ratio, 28-day mortality and serious adverse events all line up.
Protein C and PAI-1 combined genotype

• IRP+/+ patients have $\downarrow$ mortality and $\downarrow$ SAEs when treated with rhAPC.

• IRP-/- patients do not have $\downarrow$ mortality and have $\uparrow$ SAEs when treated with rhAPC.

• Maybe IRP+/+ patients should be treated with rhAPC.

• Maybe IRP-/- patients should not be treated with rhAPC
Variation In Thrombosis-Related Genes And Sepsis Outcomes

Keith R. Walley, MD
Critical Care Medicine
St. Paul’s Hospital
Heart + Lung Institute
University of British Columbia
Vancouver, BC, Canada

Disclosure: Co-founder of Sirius Genomics
Outline

• Pharmacogenomics / coagulation pathway
  – warfarin
• Severe sepsis
  – Fibrinogen
  – Protein C
  – PAI-1
• Pharmacogenomics
  – Combination Protein C / PAI-1 genotype
WARFARIN: VKORC1
(vitamin K epoxide reductase complex 1)

Pharmacogenomics

- Genotyping VKORC1 and CYP2C9 may be helpful in estimating warfarin dose
- Could genotyping in coagulation pathway genes be helpful in making therapeutic decisions in sepsis patients?

Genomics of Sepsis

### Fibrinogen-β in Sepsis
Haplotype Tag SNPs

Relative position in the Fibrinogen-β gene

<table>
<thead>
<tr>
<th>Position</th>
<th>Hap 1</th>
<th>Hap 2</th>
<th>Hap 3</th>
<th>Hap 4</th>
<th>Hap 5</th>
<th>Hap 6</th>
<th>Hap 7</th>
<th>Hap 8</th>
<th>Hap 9</th>
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<th>Hap 12</th>
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</table>

Fibrinogen-β haplotype is evolutionarily distant

- This haplotype is associated with increased transcription (-455A) and high fibrinogen levels.
- Associated with stroke, MI, venous thromboembolism
Fibrinogen-β haplotype is associated with increased survival

OR = 0.66, 95% CI = 0.46–0.94, p = 0.02
Protein C in Sepsis
Indicated Population, APACHE II ≥ 25

Log-rank test (http://www.xigris.com/360-clinical-trials.jsp#XIGRIS3)
Protein C Haplotypes

-1654 -1641
Protein C Haplotypes

Protein C SNP
rs2069912
Derivation: Risk of Death Analysis
SPH Severe Sepsis Cohort: PROC rs2069912

![Bar chart showing 28-day mortality (%)](image)

- CC/CT: 40%
- TT: 30%

$p = 0.015$
Variation In Thrombosis-Related Genes And Sepsis Outcomes

- Warfarin → pharmacogenomics
- Sepsis: Fibrinogen-β related to outcome
- Combination protein C and PAI-1 genotype in 3 cohorts.
  - Efficacy: Association of rhAPC in +/+ with decreased mortality.
  - Safety: Association of rhAPC in -/- with increased serious adverse events.
  - Biological Plausibility: PAI-1 / ProteinC levels.

Could this be used for therapeutic decisions?
Co-investigator
Jim Russell

Databases
VASST Investigators and coordinators
PROWESS Investigators
  Eli Lilly
  G Bernard
  J-L Vincent

Funding
Heart & Stroke Foundation
CIHR
Michael Smith Foundation
Sirius Genomics Inc.

People
Cheryl Holmes
Lauralynn MacIntyre
Ainsley Sutherland
Dave Shaw
Sanjay Manocha
Horatio Groshaus
Anan Wattanathum
Tony Gordon
Hugh Wellman
Taka Nakada
Emily Nakada
Katherine Thain
Simone Thair
Melissa McConechy
Lynda Lazosky
John Boyd
Meta-analysis of IRP genotypes

IRP +/+ (p < 0.0001)*

IRP +/- (p=0.33)*

IRP -/- (p=0.75)*

*meta-analysis p-values estimated using DerSimonian-Laird method (1986)
BLEEDING Serious Adverse Events (SAEs) and Adverse Events (AEs) by IRP Genotype

Within genotype
- Placebo: p=0.029, p=0.15, p=0.47
- Xigris: p=0.13, p=0.24, p=0.028

Between genotypes
- Placebo: p=0.24, p=0.99
- Xigris: p=0.66, p=0.19
Bleeding Serious Adverse Events (SAEs) by IRP Genotype

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Base</th>
<th>IRP +/+ (P = 0.029)</th>
<th>IRP +/- (P = 0.512)</th>
<th>IRP -/- (P = 0.465)</th>
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<tr>
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<td>1/138</td>
<td>7/124</td>
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<td>P</td>
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- Placebo
- Xigris
Bleeding Adverse Events (AEs) by IRP Genotype

<table>
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<tr>
<th>IRP Genotype</th>
<th>Placebo</th>
<th>Xigris</th>
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<tr>
<td>IRP +/+</td>
<td>33/138</td>
<td>41/124</td>
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<tr>
<td>IRP +/-</td>
<td>40/178</td>
<td>57/204</td>
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<tr>
<td>IRP -/-</td>
<td>5/38</td>
<td>12/33</td>
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</table>

Interaction:
- IRP +/+ (P = 0.13)
- IRP +/- (P = 0.24)
- IRP -/- (P = 0.028)
Thrombotic Serious Adverse Events (SAEs) by IRP Genotype

Interaction: Base

- IRP +/+ (P = 0.34) - Placebo: 7/138, Xigris: 3/124
- IRP +/- (P = 0.59) - Placebo: 8/178, Xigris: 7/204
- IRP -/- (P = 0.095) - Placebo: 0/38, Xigris: 3/33
Thrombotic Adverse Events (AEs) by IRP Genotype

- IRP +/+ (P = 0.82)
  - Placebo: 12/138
  - Xigris: 9/124

- IRP +/- (P = 1)
  - Placebo: 15/178
  - Xigris: 17/204

- IRP -/- (P = 0.054)
  - Placebo: 3/38
  - Xigris: 9/33

Interaction: Base

P = 0.76

P = 0.05
Medical Challenge of Sepsis

- Leading cause of death in adult ICUs
- Estimated 150K - 200K deaths / year in USA\(^1,2\)
- Equal to deaths after myocardial infarction\(^3\)
- Annual cost $16.7 billion\(^1\)

\(^1\) Angus et al CCM 2001,
\(^2\) Dombrovskiy et al CCM 2007
\(^3\) www.cdc.gov
# Role of Heredity in Infectious Diseases

<table>
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<th>Parents</th>
<th>Relative Risk of Adoptee Adults</th>
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<td>Cancer</td>
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<td>Infectious Disease</td>
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Polymorphisms of Protein C (and Protein C pathway genes) are associated with altered response to administration of Xigris in severe sepsis.

1. Sorensen TI et al. NEJM 1988; 318: 727
Proposed pathways for APC activity

Adapted from Mosnier LO et al. Blood 2006 Nov 16
Coagulation Cascade in Sepsis
### Serious Adverse Events (SAEs) in IRP-/- patients in PROWESS

All APACHE II scores

<table>
<thead>
<tr>
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<th><strong>Control</strong> (4 patients)</th>
<th><strong>rhAPC</strong> (14 patients)</th>
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<td>Major SAE</td>
<td>2 events</td>
<td>16 events</td>
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<tr>
<td>Minor SAE</td>
<td>3 events</td>
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