INVASIVE ASPERGILLOSIS: ELUCIDATION OF GENETIC RISK FACTORS FOR DISEASE

Aimee K. Zaas, MD
Duke University Medical Center, Durham, NC, USA
Thursday, February 23, 2006, 8:40 - 9:00 am

Aspergillus fumigatus is a common and deadly pathogen in immunocompromised hosts. Invasive aspergillosis (IA), typically caused by *A. fumigatus* (AF), is the most common filamentous fungal infection following bone marrow transplantation, occurring in approximately 10% of allogeneic bone marrow transplant recipients. Other high risk groups include solid-organ transplant recipients as well as patients receiving chronic corticosteroid therapy. Research over the last several years has provided a better understanding of the epidemiologic risk factors for IA; however, the currently identified risk factors explain only a minor component of the susceptibility to IA among immunocompromised hosts. It has only recently been recognized that susceptibility to IA is greatly influenced by host genetic background. Polymorphisms in genes regulating innate and adaptive immune function are likely important determinants of host susceptibility to fungal infections and may become critically important during times of immunosuppression. Studies using both animal models of IA and human cohorts are attempting to elucidate the host genetic contribution to IA susceptibility. These studies are aimed at identifying host genetic polymorphisms that confer susceptibility or resistance to IA. Studies using animal models have focused on candidate genes, utilize positional cloning, database driven haplotype mapping as well as gene expression studies. In addition, human cohort studies are using genetic association studies to correlate clinical outcomes with genetic background. Understanding the role of host genetics in this complex phenotype can lead to numerous clinical benefits and hopefully improved outcomes. An understanding of genetic susceptibility profile will allow for the implementation of specific prophylaxis strategies for high risk patients, improved donor-recipient selection, and potentially novel therapies for IA.

Risks for Invasive Aspergillosis: The Role of Host Genetics

Aimee K Zaas, MD
Assistant Professor of Medicine
Division of Infectious Diseases and International Health
Duke University Medical Center
Durham, NC USA

It's the Host, Not the Fungus?

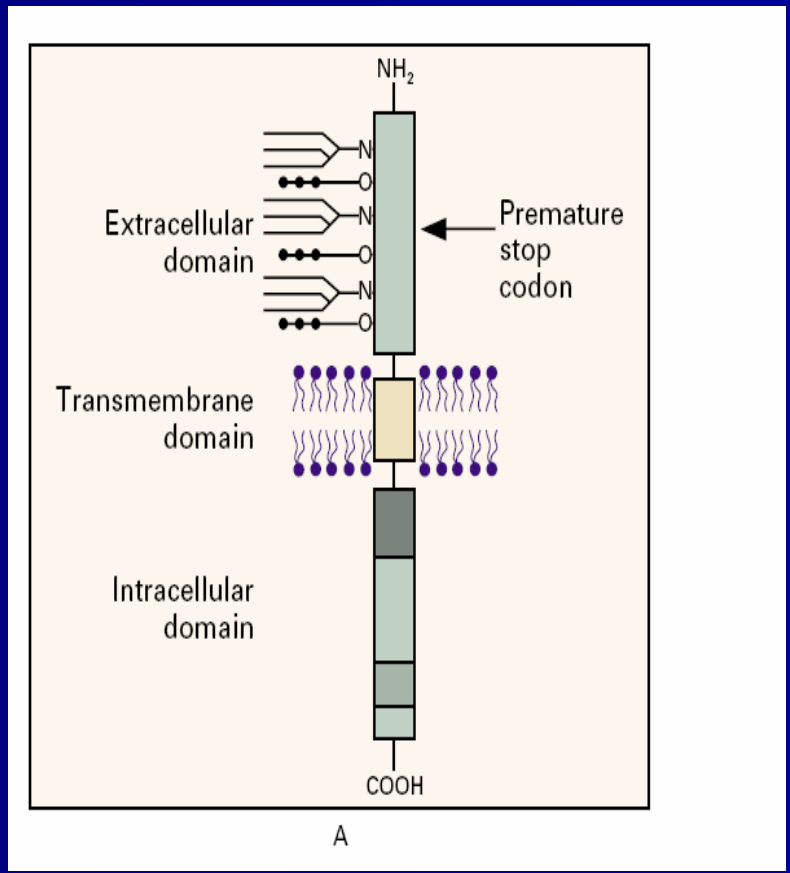
- Key observation:
  - Similar patients experience vastly different outcomes
- Hypothesis:
  - Variance in susceptibility and outcome are due to host genetic differences
- Can we use genetics to:
  - Risk stratify patients?
  - Predict risk of poor outcome?
  - Individualize prophylaxis and treatment?
  - Develop targeted interventions?

Do Genetics Determine the Risk of Infection?

- Genetic background affects risk for many diseases
  - Cardiovascular disease, malignancy, dementia
- HSCT and SOT already use genetics to maximize outcome of transplant
  - engraftment/rejection
- Increased susceptibility to infections with different genetic backgrounds
  - independent of transplantation status
- Unique to transplant: consider donor and recipient genetic background

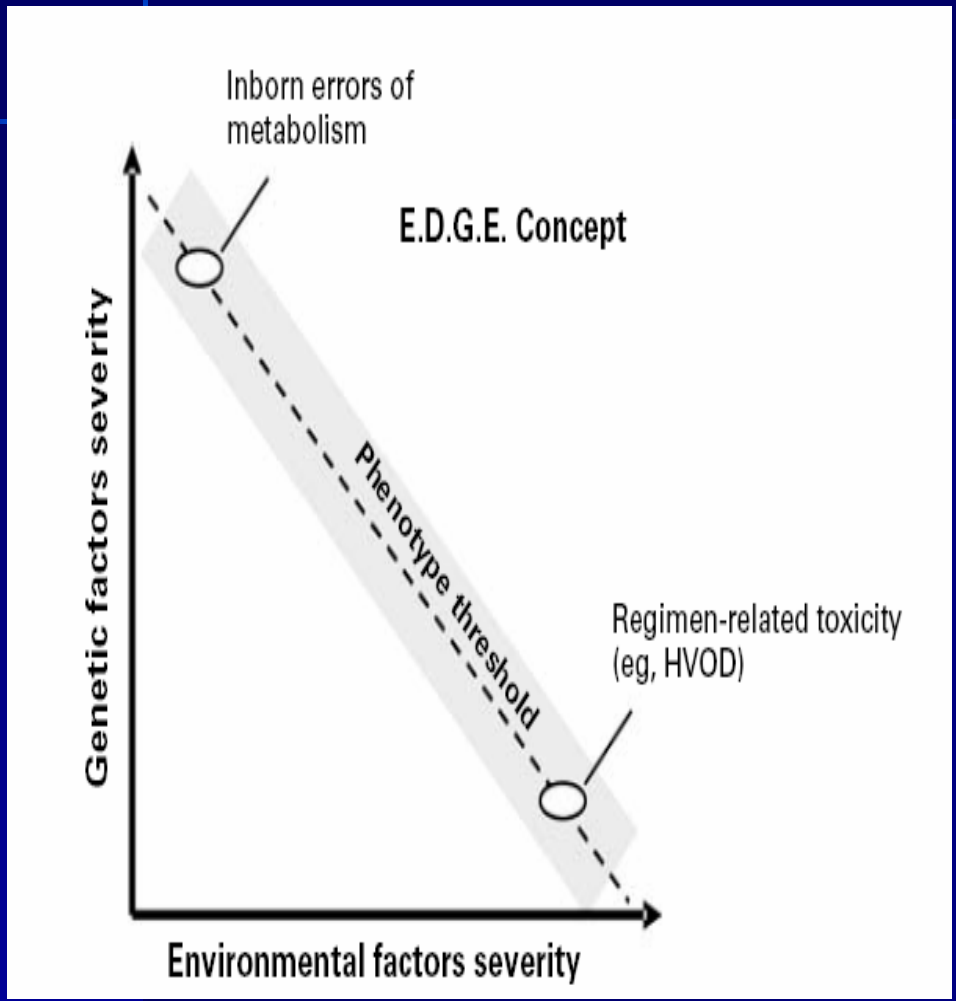
Proof of Concept: Genetic Variation Affects Infection Susceptibility

- Single gene:
  - Malaria susceptibility decreased in sickle cell trait
- Polymorphic variation:
  - Interferon-γ receptor mutations and mycobacterial susceptibility



Newport MJ, *et al.* NEJM 1996; 335:1941-9

Role of Environment



- **EDGE:**
  - *e*nvironmentally *d*etermined *g*enetic *e*xpression
  - Concept that subtle genetic variation may cause phenotypic differences under stress conditions

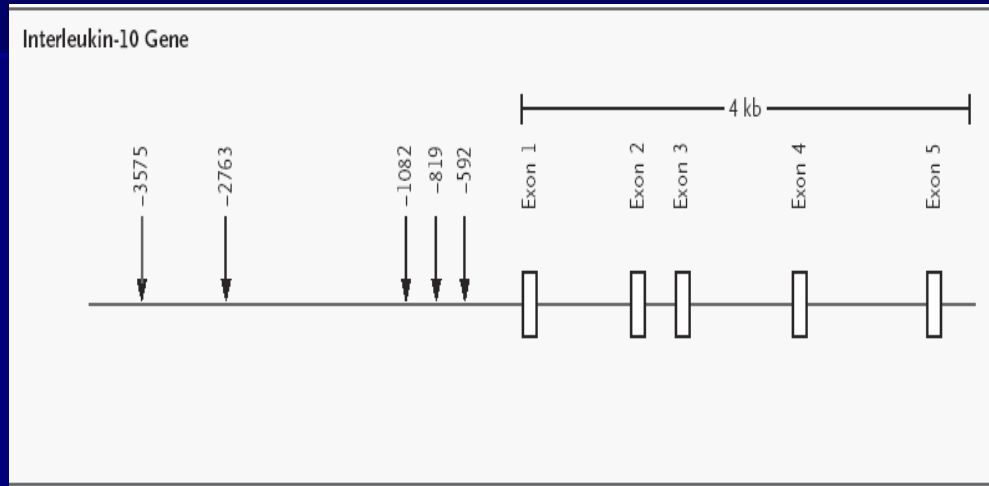
Kallianpur A. *Bone Marrow Transplantation* 2005;35:1-16.

Key Terminology

- **Genetics:**
  - study of genes function relative to disease
- **Genomics:**
  - Evaluating genes as a dynamic system over time ("transcriptome") to determine how they interact as a system with the environment
- **Phenotype:**
  - observable characteristics produced by genotype interacting with the environment
- **Linkage:**
  - Greater association in inheritance of 2+ genes than is expected OR analysis of pedigree tracking of a gene through a family
  - not particularly applicable in transplant medicine
- **Association:**
  - Looking at genes/variations in 2 groups (case/control; high responder/low responder) to establish a relationship with a phenotype

Key Terminology

- **Single nucleotide polymorphism (SNP):**
  - DNA sequence variation in a single nucleotide
  - Useful when occur in >1%
  - wild type codon / position / coding change
    - "A275C"
    - "C-59T"
- **Allele:**
  - form of gene; "wild-type" or "variant"
- **Haplotype:**
  - clusters of genetic variants that are inherited as a unit on the same chromosome



Interleukin-10 Promoter Haplotype

Distal Region		Proximal Region			Haplotype Frequency
-3575	-2763	-1082	-819	-592	%
T	C	A	T	A	28
T	C	A	C	C	28
T	C	G	C	C	6
A	C	G	C	C	6
T	A	G	C	C	4
A	A	G	C	C	29

Lin MT, *et al.* NEJM 2003; 349:2201-10.

Key Terminology

- **Candidate Gene:**
  - Gene selected for study in a particular disease process due to "biologic plausibility" or preliminary data

- **Positional Candidate Gene:**
  - Gene selected for study as a result of experiments locating a particular region on a chromosome

Invasive Aspergillosis: Candidate Gene Studies

- Biology: Innate immune system is main line of host defense against IA
- Many cytokines, chemokines, receptor families to evaluate
- Ideal system for testing individual candidates: “knock-out” mouse

Candidate Genes: An Association Study "How To"

- Is my favorite gene a biologically plausible candidate?
- Are polymorphic variants known in my population?
- Is the function of polymorphic variants established? If not, can I establish it?
- Do I have enough patients/events?

- In transplant setting, am I measuring the right genotype?

Candidate Gene: The IL-10 Story

- IL-10: pushes towards Th2 phenotype; deleterious in aspergillosis
- *In vitro:* IL-10 reduces mononuclear cells ability to damage *Aspergillus* hyphae
- *In vivo:* increase in resistance to IPA after neutralization of IL-10
- IL-10 KO mouse: less susceptible to IA than WT controls

Roilides E, *et al.* J Immunol 1997;158:322-9; Cenci E, *et al.* JID 1998;178:1750-60; Clemons KV, *et al.* Clin Exp Immunol 2000;122:186-91.

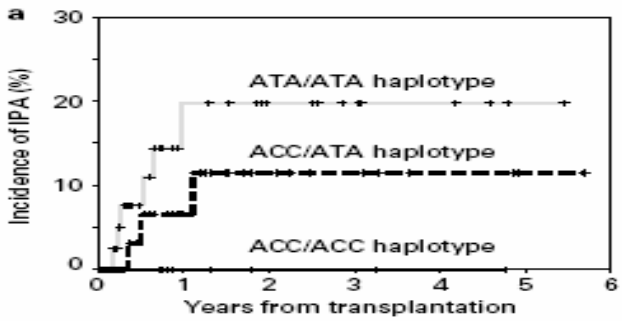
Polymorphic Variants of IL-10

- NCBI build 35.1: 800 human SNPs in IL-10 reported; 3 promoter SNPs; 2 microsatellite (CA)n repeats; 27 NS-coding change SNPs
- IL-10 inducibility is variable → genetic component related to promoter SNPs
- Polymorphisms affect susceptibility to sepsis, GVHD, outcome from BMT

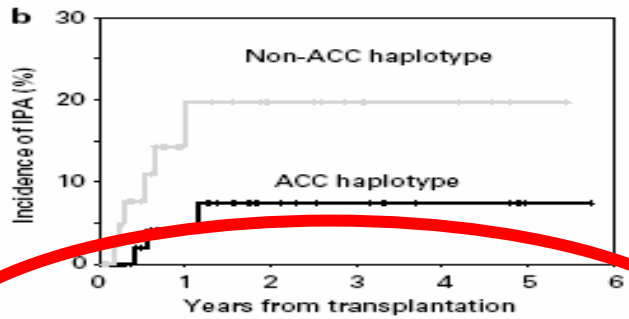
Stanislova SA, *et al.* Intensive Care Med 2006; 134:260-6; Eskdale J, *et al.* PNAS 1998; 95:946-70; Lin MT, *et al.* NEJM 2003; 349:2201-10.

Do IL-10 promoter haplotypes associate with IA?

- 105 HSCT recipients prospectively studied
  - 75% HLA match sib
  - 15% HLA match unrelated
  - 11% HLA mismatch sib
- Median f/u 292 days
  - 9 cases IA
- ACC haplotype dominant/protective



Haplotype	No of pts	IPA at 2 years (%)	*P*-value
ACC/ACC	0/12 (0%)	0	0.2734
ACC/ATA	3/49 (6.1%)	11.5±6.4	
ATA/ATA	6/44 (13.6%)	19.7±7.7	



Haplotype	No of pts	IPA at 2 years (%)	*P*-value
ACC	3/61 (4.9%)	7.0±4.9	0.0307
Non-ACC	6/44 (13.6%)	19.7±7.7	

Seo KW, *et al.* Bone Marrow Transplantation 2005;36:1089-95.

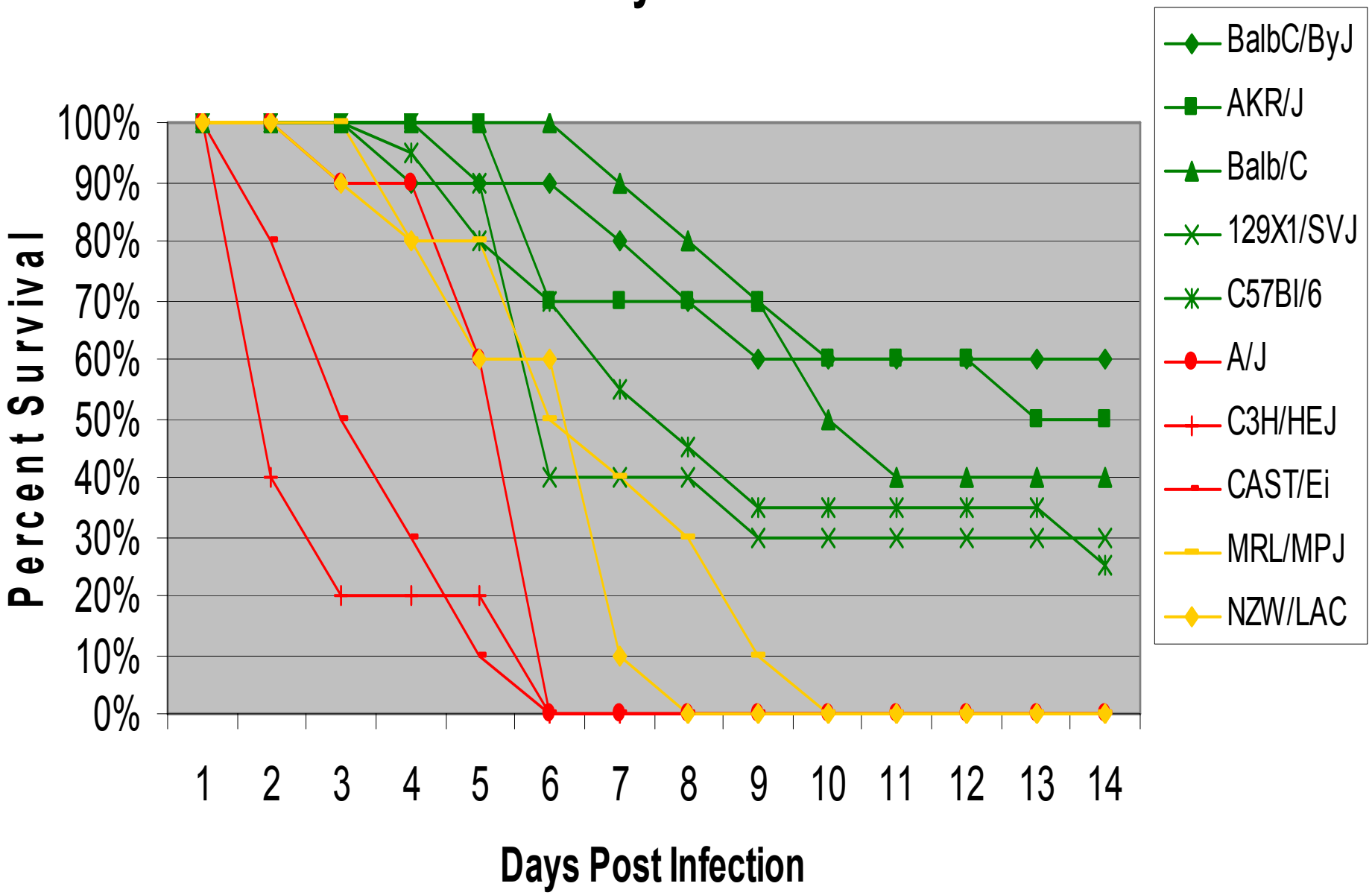
How Well does this Study Do?

- Biologically plausible candidate?
- Polymorphic variants known (in my population)?
- ? Function of polymorphic variants established? If not, can I establish it?
- ? Do I have enough patients/events?
- ? In transplant setting, am I measuring the right genotype?

From Mouse Genes to Human Cohorts

- Traditional positional cloning to select candidate gene → validate in human cohorts
  - Pro: no bias about what is a candidate
  - Con: years to locate candidate gene

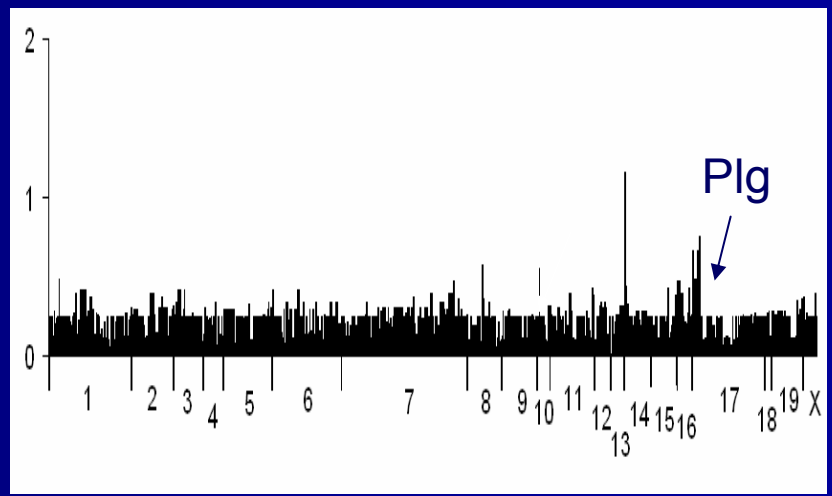
- *"In silico"* haplotype associated mapping*:* Uses available mouse genome data to quickly locate candidate genes
  - Pro: faster time to validate
  - Con: limited by available mouse genome data; best for very specific phenotypes


14-Day Survival
Percent Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Days Post Infection
BalbC/ByJ
AKR/J
Balb/C
129X1/SVJ
C57Bl/6
A/J
C3H/HEJ
CAST/Ei
MRL/MPJ
NZW/LAC

Haplotype Associated Mapping: Genotype to Phenotype

- *In silico* mapping used to evaluate phenotype 14-day survival

Score	Haplotype	Chr.	Position	#SNPs	Symbol	CB	CD	HP	KD	LV	LN	PF	QD	SC	SP
0.859		14	13.333788~13.334710	10	Thrb										
1.319		17	11.549847~11.800883	22	Plg										
1.519		17	10.558926~10.559185	6	Park2										
1.519		17	11.542105~11.543439	8	Plg										
1.746		8	82.557754~82.563585	24	Ucp1										
1.786		10	6.637267~7.334629	6	Oprm										
2.071		17	11.508305~11.510066	10	nb:Plg										
2.071		17	11.514254~11.541214	75	Plg										
2.096		1	73.566884~73.567885	6	nb:Igfbp5										



Zaas AK, *et al.* International Conference on Fungal Genetics; Copenhagen 2004

Candidate Gene Validation

- NS-coding change SNP (G110S) present in PLG in susceptible strains
  - not present in resistant strains
- Ongoing validation:
  - Human HSCT cohort
  - Functional studies of PLG interaction with *Aspergillus fumigatus*
  - Functional studies of polymorphic PLG variant

Conclusions

- Individual risk for *Aspergillus* infection in the appropriate host likely has a genetic basis
- Tools are available to uncover and validate the role of genetic polymorphisms in determining disease risk
- Field is in its infancy → much validation, specific to patient type, is needed

Applications

- Select highest risk patients for most aggressive prophylaxis
- Pre-transplant profiling
  - select more ideal "donor-recipient" pairs
  - individualize immunosuppression
- Novel therapies
- **Bank DNA for the future!**

Thank You

- David Schwartz (NIEHS)
- John Perfect (Duke University Medical Center)
- Gary Peltz and Guochun Liao (Roche PaloAlto)
- Bill Steinbach (Duke University Medical Center)
- Jason Chien, Joan Clarke and Kieren Marr (FHCRC)