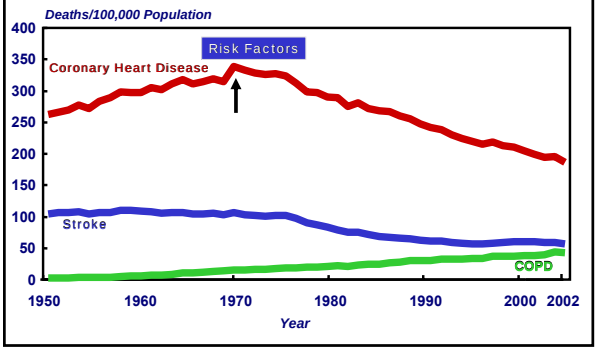
Duke Institute for Genome Science and Policy

Genomic Medicine and Cardiovascular Disease

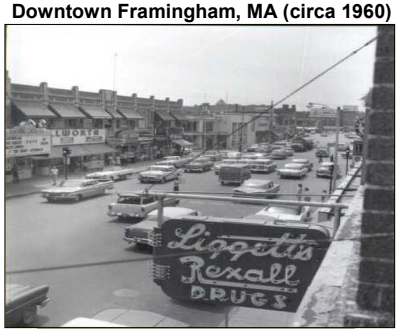
Elizabeth G. Nabel, M.D.
Director
National Heart, Lung, and Blood Institute
National Institutes of Health
March 2, 2006

Age-Adjusted Death Rates for Coronary Heart Disease, U.S., 1950-2002



Framingham Heart Study

Downtown Framingham, MA (circa 1960)



"Factors of Risk" for CVD

Annals of Internal Medicine

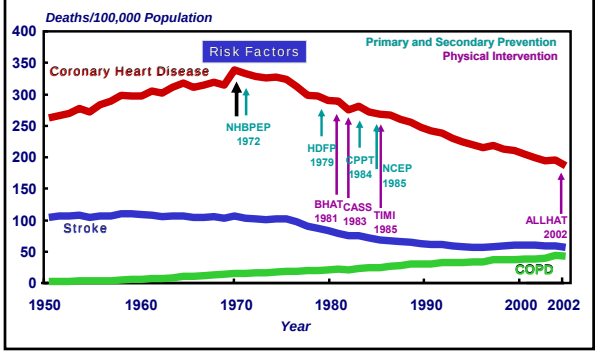
Factors of Risk in the Development of Coronary Heart Disease—Six-Year Follow-up Experience

W. Kannel et al.

November 1961
Vol. 55, No.1

- High Blood Pressure
- Increased Cholesterol
- Smoking
- Diabetes
- Family History
- Male Gender

Age-Adjusted Death Rates for Coronary Heart Disease, U.S., 1950-2002

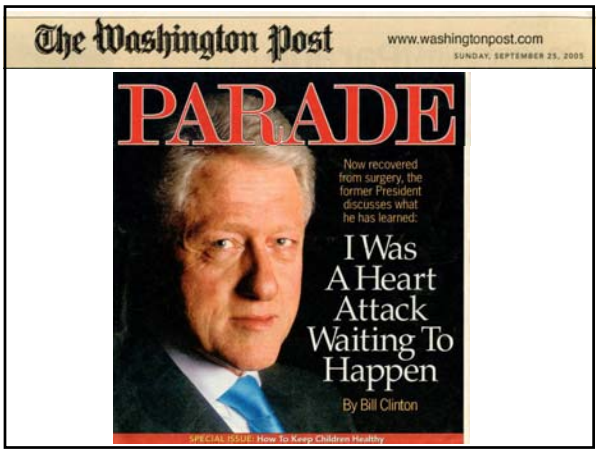


Eisenhower's Billion-Dollar Heart Attack — 50 Years Later

Franz H. Messerli, M.D., Adrian W. Messerli, M.D., and Thomas F. Lüscher, M.D.

N ENGL J MED 353;12 WWW.NEJM.ORG SEPTEMBER 22, 2005

"Wall Street panicked, the DOW Jones had dropped by 6 percent – a paper loss of $14 billion, the largest decline since the crash of 1929"



Future Directions in CV Research

Early identification of subclinical CVD

- **Phenotype Markers**
  - **Biomarkers: CRP**
  - **Imaging**
- **Genotype Markers**
  - **Familial hypertrophic cardiomyopathy**
  - **Warfarin resistance and sensitivity**

Whole Genome Association Approach to Common Disease: The View from 2006 (the HapMap Era)

- **Identify an optimum set of 300,000 tag SNPs**
- **Collect 1000 cases and 1000 controls**
- **Genotype all DNAs for all SNPs**
- **That adds up to 600 million – not 20 billion - genotypes**
- **And, genotyping has dropped to $0.005, so that's ~$3 million for each disease**

The First HapMap Success Story: Age-Related Macular Degeneration

Complement Factor H Polymorphism in Age-Related Macular Degeneration

Robert J. Klein,[1] Caroline Zeiss,[2*] Emily Y. Chew,[3*] Jen-Yue Tsai,[4*] Richard S. Sackler,[1] Chad Haynes,[1] Alice K. Henning,[5] John Paul SanGiovanni,[3] Shrikant M. Mane,[6] Susan T. Mayne,[7] Michael B. Bracken,[7] Frederick L. Ferris,[3] Jurg Ott,[1] Colin Barnstable,[2] Josephine Hoh[7†]

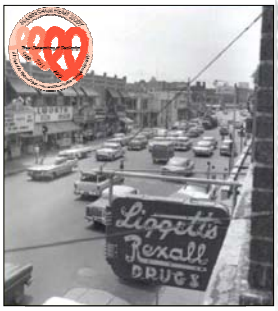
A Tyrosine to Histidine variant in codon 402 of the Complement Factor H gene accounts for approximately half of the attributable risk of AMD in older adults

NHLBI Genetics and Genomics Studies

- FHS Genetic Research Study
- Genome-Wide Association Studies
- Women's Health Initiative Genomic Studies
- Larger-Scale Genotyping of NHLBI Coharts
- Genetic Alpaca Improvement Network (GAIN)
- Genes and Environment Initiative (GEI)

Framingham Heart Study

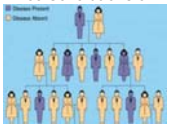
Downtown Framingham, MA (circa 1960)



- "Major Risk Factors" for Heart Attack, Stroke, other Cardiovascular Diseases
  - High blood pressure
  - High cholesterol
  - Cigarette smoking
  - Diabetes mellitus
  - Parental or sibling history
  - Obesity
- Important New Markers of Risk
  - C-reactive protein & other "bio"markers
  - Metabolic syndrome
  - Thick heart muscle on ultrasound
  - Artery plaques on CT & MRI scans
  - Genetic markers

Framingham Heart Study: Major Contributions to Come

Three Generations of:



- Men and Women
- Key Physiological Measures
  - At the Same Age
  - Obtained Over Decades
- Genetic Material for 9000 of Them

Framingham Heart Study Genome-Wide Research Study 2006-2007

- Study Community Men and Women
  - Hundreds of disease measures
  - 500,000 genetic tests per person
- Informed Consent & Ethics Oversight
- Rapid Conduct of Genetic Research
  - Database by National Library of Medicine
  - Share data broadly with scientists
  - Stimulate gene discovery research:
    - Heart and lung disease and stroke
    - New & major CVD risk factors
    - Other diseases: e.g., lung and bone
- Translate Research to Better Understand, Prevent & Treat Disease

Genome Wide Association Studies

- Design and Analysis of Genome-Wide Association Studies
  - RFA being funded now
  - NHLBI lead; NHGRI, NIEHS, NCI, NIGMS are participants

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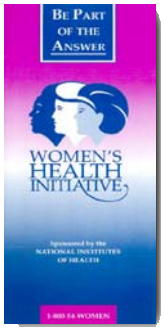
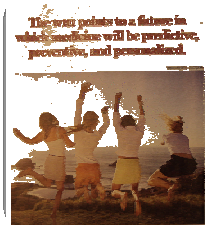
- Genome Wide Association Studies to Identify Genetic Components that Relate to Heart, Lung, and Blood Disorders
  - RFA, application due April, 2006
  - Start date September, 2006

The Best is Yet to Come!



The greatest potential of the study lies in these billions of data points drawn from two questions of women representing all of America's diversity.



Large-Scale Genotyping of NHLBI Cohorts

- **High-throughput genotyping for**
  1. **Candidate gene association studies in up to 50,000 participants from multiple NHLBI cohorts**
  2. **Genome-wide association study in roughly 500 cases and 1000 controls**

Large-Scale Genotyping of NHLBI Cohorts

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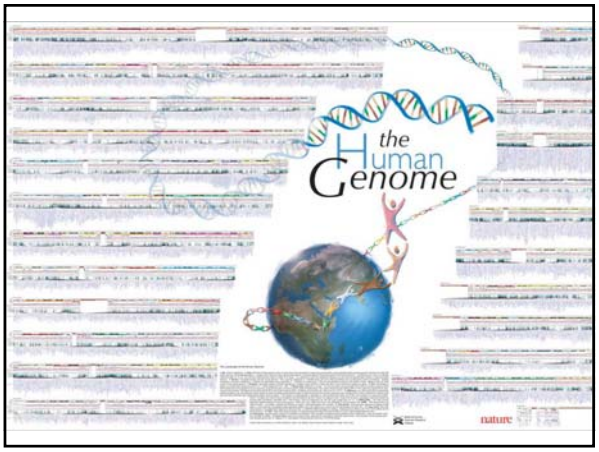
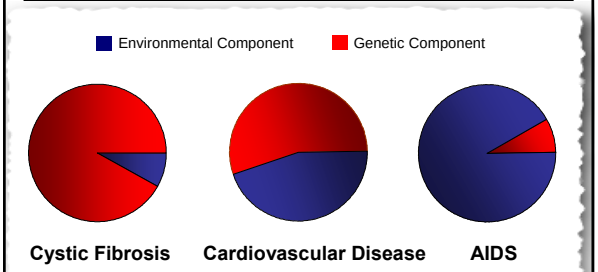
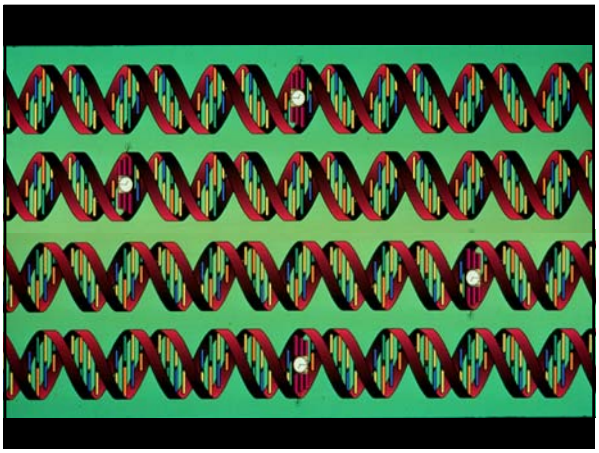
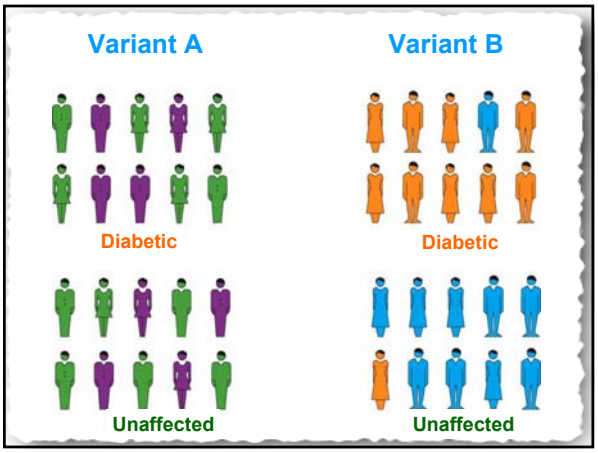
- **Contract in final stages of negotiation**



Virtually All Diseases (Except Maybe Trauma) Have a Genetic Component







Sequence from Chromosome 7

Three single nucleotide polymorphisms (SNPs) are present.

Whole Genome Association Approach to Common Disease: The View from 2002

- Identify all 10 million common SNPs
- Collect 1000 cases and 1000 controls
- Genotype all DNAs for all SNPs
- That adds up to 20 billion genotypes
- At 50 cents a genotype, that's $10 billion for each disease

Sequence from Chromosome 7

Are the SNPs correlated with their neighbors?

These Three SNPs Could Theoretically Occur in Eight Different Haplotypes

…C…A…A…
…C…A…G…
…C…C…A…
…C…C…G…
…T…A…A…
…T…A…G…
…T…C…A…
…T…C…G…

But in Practice, Only Two are Observed

…C…A…A…
…C…A…G…
…C…C…A…
…C…C…G…
…T…A…A…
…T…A…G…
…T…C…A…
…T…C…G…

The Genetic Association Identification Network (GAIN)

- Provides genotyping for a number of longstanding NIH case-control studies and makes data available immediately to all appropriate researchers
- The Foundation for the NIH receives funds from private sector donors and manages the project
- No funder gets special access to the data
- Major initial funding from Pfizer and Affymetrix
- See www.fnih.org for details

The Genes and Environment Initiative (GEI)

- Proposed in the President's budget for FY07 (must be approved by Congress)
- Builds upon longstanding NIH investment in developing case-control studies
- Provides genotyping for dozens of such case-control studies, with immediate data access
- Develops innovative technologies to measure environmental exposures, diet, and physical activity

GEI: Budget for Whole Genome Association and Environmental Technology Development

Dollars in Millions

	FY 06 NIH	FY 07 NIH	FY 07 President's Budget
WGA	15	20	26
Envir Tech	4	8	14

Why These Initiatives Now?

- Because HapMap and reduced genotyping costs make it possible to identify the major genetic risk factors for common diseases like diabetes, cancer, heart disease, hypertension, bipolar illness, asthma, Alzheimer's disease, osteoporosis, and many other diseases in the next 2 - 5 years
- And, because innovative technologies would allow us to measure environmental exposures, diet, and physical activity as well as we can already measure genotypes

Discovery of Hereditary Factors in Common Disease Will Allow

- "Predictive, preemptive, personalized medicine"
  - New ways to predict individual risk for common diseases
  - New and individualized ways to prevent common diseases
  - New and individualized ways to treat common diseases

Issues to Consider in Large Scale Projects that Relate Genotype to Phenotype

- What is the optimum standard for data access?
- What level of de-identification provides adequate confidentiality protection to participants without damaging the science?
- When should individual results be returned?
- What is adequate informed consent?

Data Access -- Issues

- Immediate and unfettered access to all qualified users provides maximum opportunity for scientific progress
- But must protect confidentiality of research participants
- And must recognize need of investigators for academic recognition

Data Access – Possible Model

- Data immediately placed in a central database
- Data Access Committee reviews user applications
- User must:
  - provide a credible research plan
  - agree not to try to identify individuals
  - agree not to submit publications for x months
  - agree not to distribute data to third parties
  - show evidence of training in human subjects research (is IRB approval required?)
- A Data and Participant Protection Monitoring Board assesses adequacy of protections

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De-identification -- Issues

- Must all links to the individual be irreversibly broken?
- If not, what personal identifiers must be removed before placing data in central database?
- Isn't extensive genotype or sequence data personally identifying anyway?

De-identification – Possible Model

- Original investigator retains links to individual if that will enhance value of research study (often true)
- All data submitted to central database is stripped of personal identifiers (HIPAA list?)
- User agrees not to attempt to identify individuals

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Returning Results -- Issues

- Many research projects are predicated on never giving back genetic results
- But if samples are not irreversibly anonymized, and information of compelling clinical utility is discovered, is it ethical not to provide that?
- What should be the threshold for disclosure?
- How can CLIA standards be maintained?
- Who provides counseling?
- Who pays?

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Consent -- Issues

- Local IRB must approve protocol – but guidelines for this situation are not entirely clear
- Can consents for earlier studies ever be adequate for open access model?
- Is re-consent really practical?
- When is a waiver justified?

Consent – Should Include

- Use of samples for genetics/genomics
- Collection of health information
- Sharing of data widely
- Risks
  - Loss of privacy
  - Stigmatization
  - Discrimination
- Plans for disclosure of results
- Ability to withdraw samples or data

We look to a future in which medicine will be predictive, preventive, and personalized.

Biomarkers of Inflammation

Conclusions:

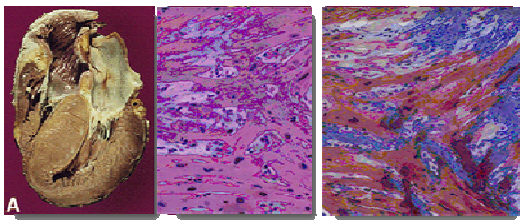
- CRP is a strong predictor of CV risk, and lowering CRP with statin therapy may be effective in the 1º and 2º prevention of coronary events.
- CRP end-points may be useful biomarkers in clinical trials, and CRP recommendations should be considered in treatment guidelines.

Familial Hypertrophic Cardiomyopathy

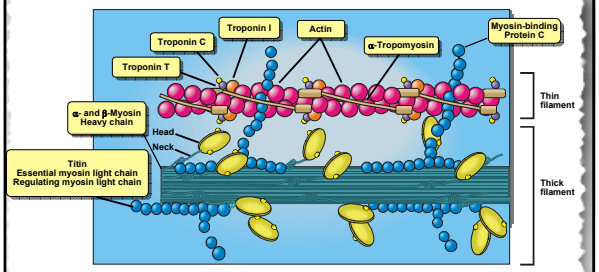
- Most common heritable cardiac disorder.
- Most frequent cause of sudden cardiac death in children and adolescents.
- 300 cardiac deaths a year in high school and college athletes in the USA; one-third of these deaths are caused by FHC.
- Sarcomeric mutations account for 75% of diagnoses in familial hypertrophy and 20% of diagnoses in elderly onset hypertrophy

Cardiomyopathies

Patterns of Cardiac Remodeling



HCM Caused by Gene Mutations in Sarcomere Proteins

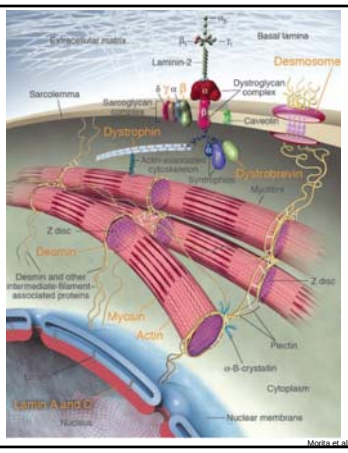


HCM Sarcomere Protein Genes

Gene Name	Abbr.	Loc.
Beta myosin heavy chain	MYH7	14q12
Myosin binding protein C	MYBPC3	11p11
Troponin I	TNNI3	19q13
Troponin T	TNNT2	1q32
Alpha tropomyosin	TPM1	15q22
Myosin regulatory light chain	MYL2	12q23-q24
Myosin essential light chain	MYL3	3p21
Actin	ACTC	15q14
Titin	TTN	2q24
Alpha myosin heavy chain	MYH6	14q12
Troponin C	TNNC1	3p21-p14

Selected HCM Non-Sarcomere Protein Genes

Gene Name	Abbr.	Loc.
HCM Associated		
Myosin light chain kinase	MYLK2	20q13
Phospholamban	PLN	6q22
Caveolin	CAV3	3p25
Glycogen Cardiomyopathy		
Protein kinase A, gamma subunit	PRKAG2	7q36
Lysosome-associated membrane	LAMP2	Xq24
Alpha galactosidase A	GLA	Xq22

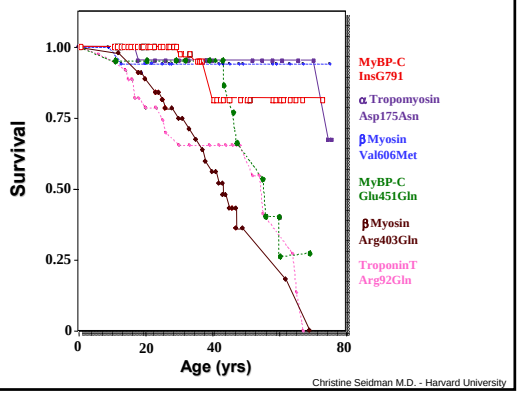


Morita et al. J Clin Invest. 2005;115:521

Selected DCM Protein Genes

Gene Name	Abbr.	Loc.
Cytoskeleton		
Lamin A/C	LMNA	1q21
Desmin	DES	2q35
Dystrophin	DMD	Xp21
Delta sarcoglycan	SGCD	5q33
Metavinculin	MVCL	10q22-q23
Sarcomere		
Beta myosin heavy chain	MYH7	14q12
Myosin binding protein C	MYBPC3	11p11
Troponin T	TNNT2	1q32

Influence of HCM Genotype on Survival



Christine Seidman M.D. - Harvard University

Paradigm Shift

Genotype patients at risk rather than waiting until 1st presentation of disease

↓

Follow genotype (+) patients, watching for early conversion of benign to hypertrophic phenotype

↓

Clinical studies and registries to guide interventions and to improve patient outcome

Translating Basic Knowledge to Clinical Practice in HCM

- Identify genetic mutations
  - Only one CLIA-certified lab in the US
  - Because of genetic variability, it may be difficult to identify mutations in *de novo* cases
  - Insurance may not cover the test, which can cost $1,000 or more.

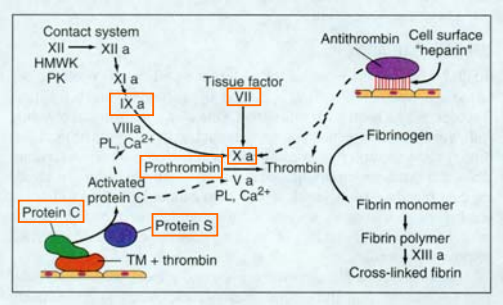
Translating Basic Knowledge to Clinical Practice in HCM

- Collect systematic clinical information
  - Registry of patients with HCM
  - Registry of sudden cardiac death
  - Registry of athletes
- Initiate intervention trials based upon genotype

Anticoagulation Therapy: Warfarin

- Indicated for the prevention of thromboembolism in atrial fibrillation, valvular heart disease, recurrent MI, stroke, and DVT
- 21.2 million prescriptions written in U.S. in 2003
- Inhibits γ–carboxylation of glutamic acid residues in Vitamin K-dependent factors: prothrombin; factors VII, IX, and X; and protein C and S.

Extrinsic and Intrinsic Coagulation Pathways



Harrison's Princples of Internal Medicine 16th Edition

Warfarin dose is influenced by:

- Dietary stores of vitamin K
- Liver function
- Co-existing medical conditions
- Concurrent medications
- Cytochrome P450 2C9 gene mutations
- *VKORC1* haplotypes (vitamin K epoxide reductase complex 1)

Therapeutic challenges:

- Safe and effective stabilization dose
- Maintenance dose

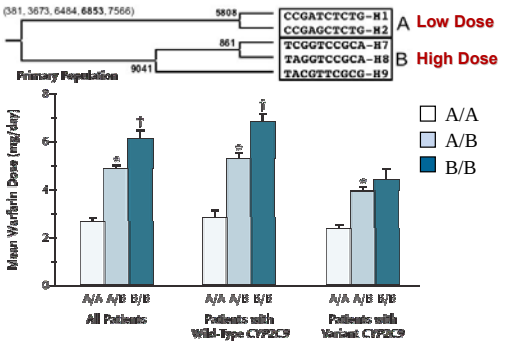
Cytochrome P450 Enzyme 2C9 (*CYP2CP*): Warfarin Sensitivity

Patients with *CYP2C9* *2 and *3 allelic variants:

- Require lower maintenance doses
- Have longer times to dose stabilization
- Present a higher risk for serious, life-threatening bleeding
- Mechanism: *CYP2C9* is responsible for the metabolic clearance of the more pharmacologically potent S-enantiomer of warfarin.

Higashi et al., *JAMA* 2002;287:1690

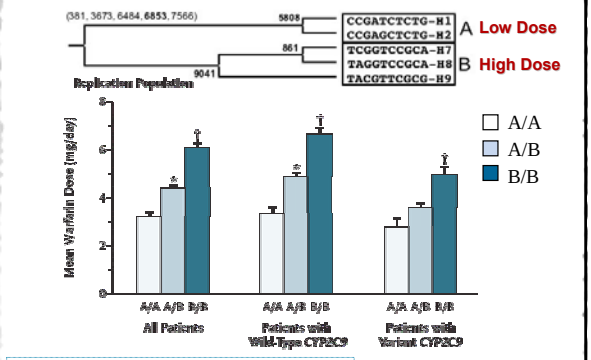
VKORC1 Haplotypes are Associated with Warfarin Dose



VKORC1 explains ~25% of warfarin dose variance

Rieder et al., *N Eng J Med* 2005; 352:2285

VKORC1 Haplotypes are Associated with Warfarin Dose

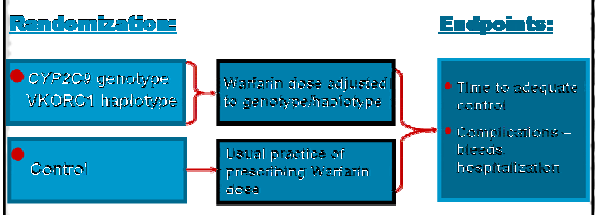


Asian Americans-higher proportion of Group A haplotypes
African Americans-higher proportion of Group B haplotypes

Rieder et al., *N Eng J Med* 2005; 352:2285

Gene Based Clinical Trial

Warfarin Resistance and Sensitivity



Hutchinson-Gilford Progeria Syndrome

Hutchinson-Gilford Progeria Syndrome

Recurrent *de novo* point mutations in lamin A cause Hutchinson–Gilford progeria syndrome

Maria Eriksson*, W. Ted Brown†, Leslie B. Gordon‡, Michael W. Glynn§, Joel Singer‖, Laura Scott‖, Michael R. Erdos*, Christiane M. Robbins*, Tracy Y. Moses*, Peter Berglund¶, Amalia Dutra*, Evgenia Pak*, Sandra Durkin§, Antonei B. Csoka#, Michael Boehnke‖, Thomas W. Glover§ & Francis S. Collins*

* National Human Genome Research Institute, and ¶ Laboratory of Viral

Eriksson et al., *Nature*, 2003

Table 1
Diseases caused by mutations in lamins A and C

Striated muscle diseases (cardiomyopathy with variable skeletal muscle involvement)
Autosomal dominant Emery-Dreifuss muscular dystrophy (no. 181350)
Autosomal recessive Emery-Dreifuss muscular dystrophy (no. 604929)
Cardiomyopathy dilated 1A (no. 115200)
Limb-girdle muscular dystrophy type 1B (no. 159001)

Partial lipodystrophy syndromes (with or without developmental abnormalities)
Dunnigan-type familial partial lipodystrophy (no. 151660)
Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and leukomelanodermic papules (no. 608056)
Mandibuloacral dysplasia (no. 248370)

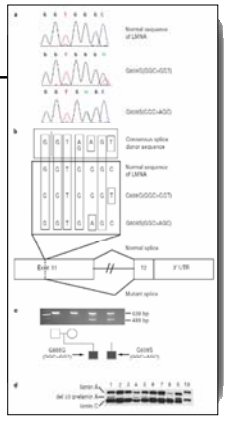
Peripheral neuropathy
Charcot-Marie-Tooth disorder type 2B1 (no. 605588)

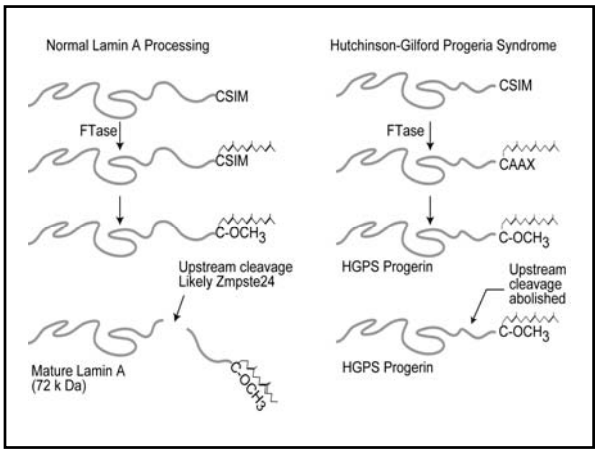
Premature aging syndromes
Hutchinson-Gilford progeria syndrome (no. 176670)
Atypical Werner syndrome (no. 277700 for Werner syndrome)

Additional information and original references can be found within ref. 5 and at the Online Mendelian Inheritance in Man database (OMIM; ref. 19). OMIM entry numbers are given in parentheses.

LMNA Gene Mutation

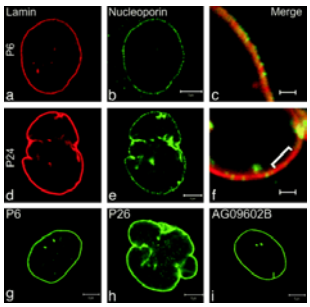
- **Single base substitution, G608G (GGC>GGT), within exon 11**
- **Activates cryptic splice site, producing a protein ("progerin") with 50 deleted amino acids (608-657) near the carboxy terminus**





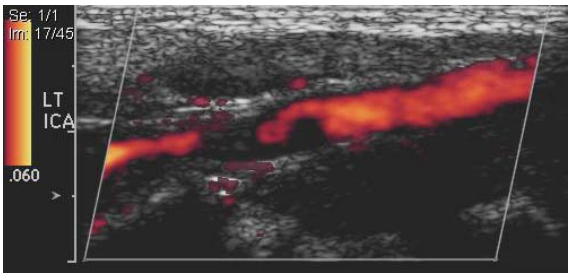
Accumulation of Progerin in the Nuclear Membrane

- Nuclear blebbing leading to:
  - Altered nuclear shape
  - Nuclear pore clustering

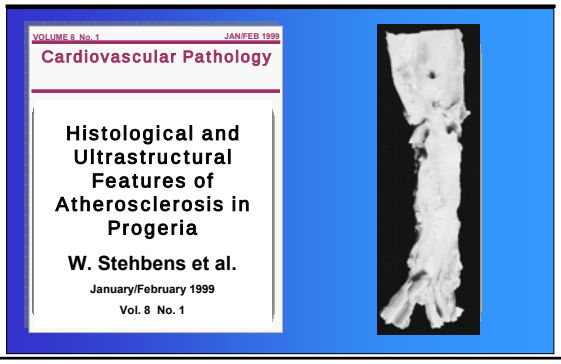


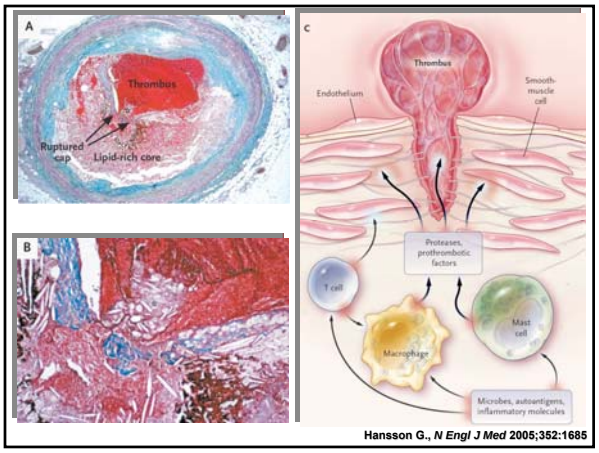
Goldman et al., *Proc Natl Acad Sci USA* 101, 8963-8968.

Complete Occlusion of the Left Common Carotid Artery in a Nine Year Old Child with HGPS



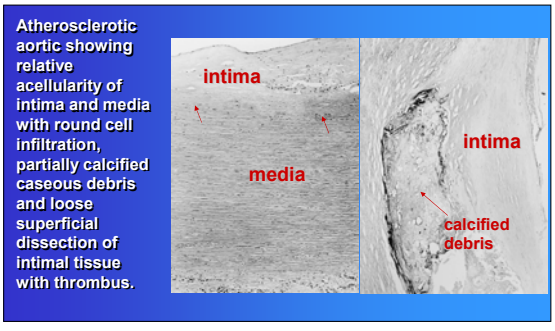
Vascular Remodeling in HGPS





Hansson G., *N Engl J Med* 2005;352:1685

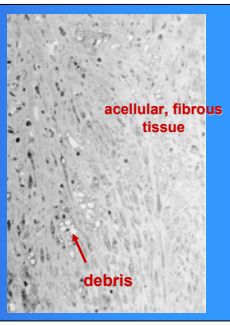
Histological Features of Vascular Remodeling in HGPS



Stehbens et al, *CardiovascPathol* 1999;8:29-39

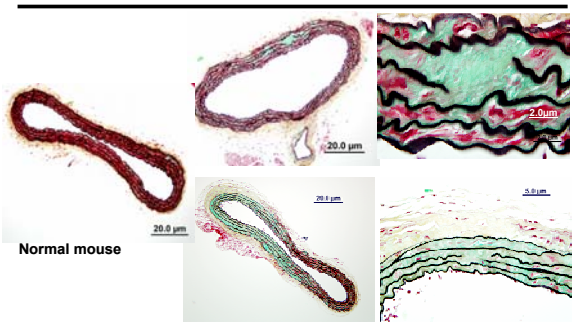
Loss of Vascular Smooth Muscle Cells and Replacement by Fibrosis

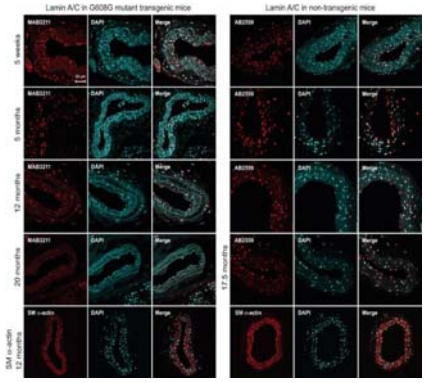
Atherosclerotic aortic intima exhibiting fibrotic tissue overlying caseous debris deep in intima.

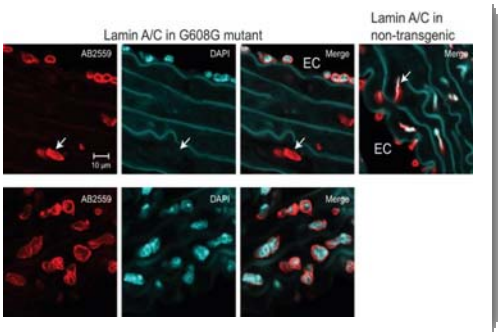


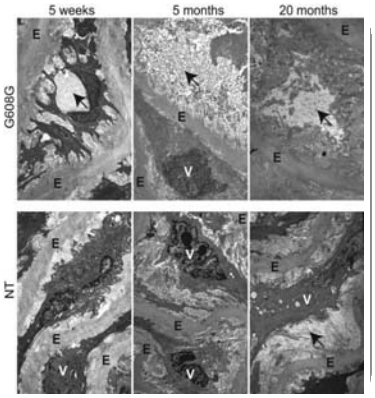
Stehbens et al, *CardiovascPathol* 1999;8:29-39

Progerin Mice Lose Vascular Smooth Muscle Cells in the Aorta and Other Blood Vessels

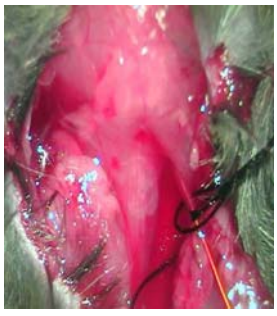






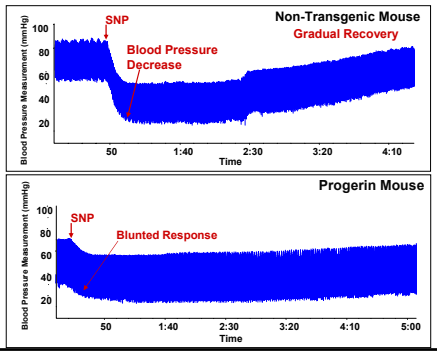


Blood Pressure Measurements

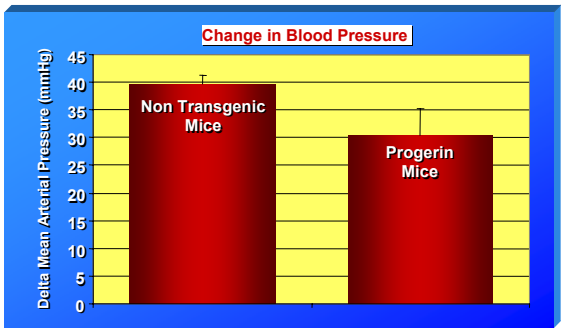


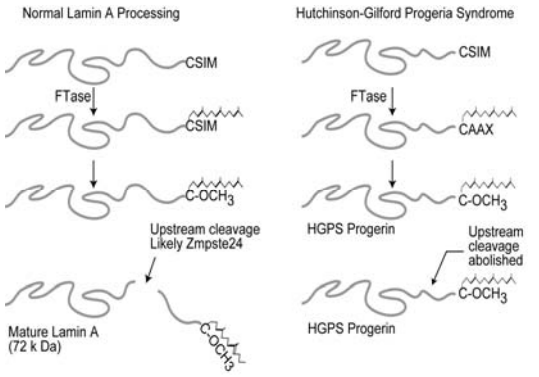
- 1 - 3% Isoflurane anesthesia
- Cannulation of the right carotid artery with a Millar conductance catheter
- 2 infusions of 0.9% saline or sodium nitroprusside 0.1 mg/kg into the left jugular vein
- Continuous measurements of blood pressure over two hours using ARIA single-Segment Pressure-Volume Conductance System

Impaired Maintenance of Blood Pressure upon Infusion of Sodium Nitroprusside (SNP) in Progerin Mice



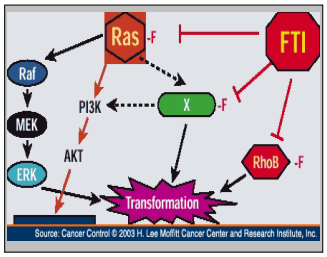
Blunting of Blood Pressure Response in Progerin Mice





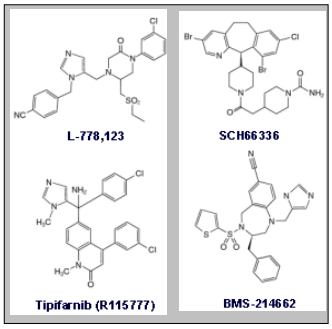
Farnesyltransferase Inhibitors (FTIs)

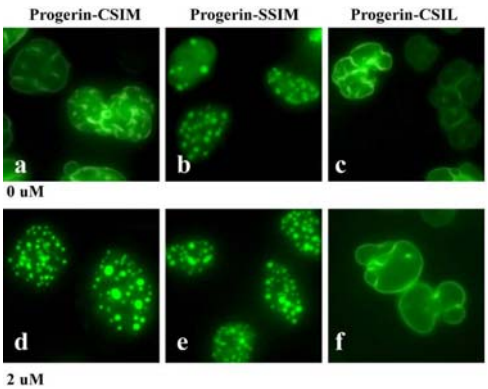
- Inhibit intracellular farnesyltransferase
- Designed in the early 1990s to inhibit the oncoprotein Ras
- Only 5 years (1993-1998) from first description to Phase I trials



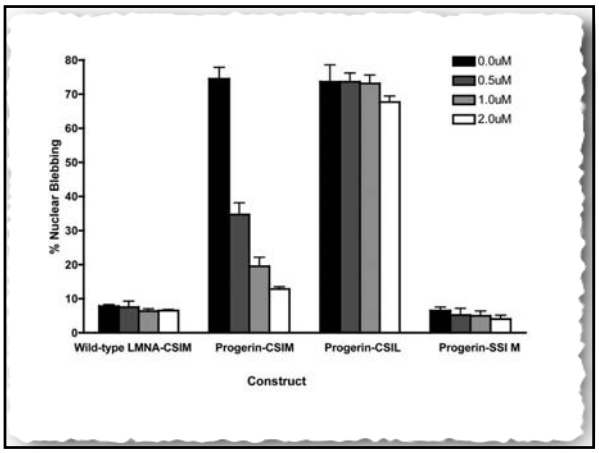
Farnesyltransferase Inhibitors (FTIs)

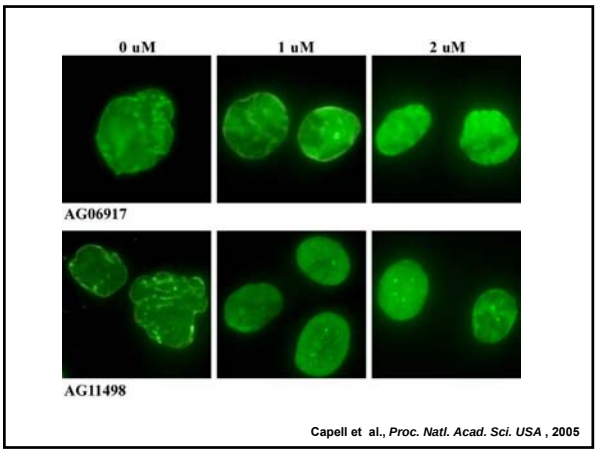
- FTIs have been shown to be well-tolerated overall in Phase I trials
- Two in Phase III clinical trials, including children
  - R115777 (tipifarnib): myelosuppression, neurotoxicity
  - SCH6636 (lonafarnib): GI effects, fatigue





Capell et al., *Proc. Natl. Acad. Sci. USA* , 2005





Capell et al., *Proc. Natl. Acad. Sci. USA* , 2005

"Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."

Sir Winston Churchill,
November, 1942