Molecular Diagnosis

In Oncology
&
Genetics

Diagnostic Molecular Pathology

- USE OF:
  - Sequence Specific INFORMATION
    - in
  - MACROMOLECULES
    - for
  - Risk identfication
  - Diagnosis
  - Prognosis
  - Prediction of response to therapy
  - Montoring therapeutic responses

Macromolecules

- Peptides/proteins
- Polysaccharides
- Polynucleotides/nucleic acids

"Nucleic Acid Diagnosis"

- Use of specific sequence information in
- nucleic acids
  - DNA and RNA
- for clinical diagnosis

Analysis Of Information In Nucleic Acids

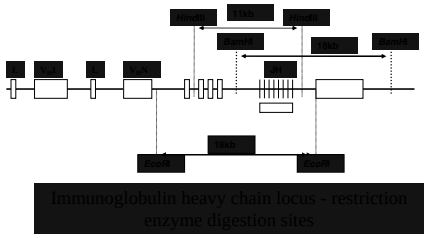
- Sequencing
- Hybridization
- Amplification
  - with specific primers
- Restriction enzyme digestion
  - Recognize specific sequences
- Electrophoretic mobility
- Translation

Molecular Oncology

- DIAGNOSITC/PROGNOSTIC INFORMATION PROVIDED BY:
  - Gross alterations in DNA content of tumors
  - Cell cycle information
  - Molecular Markers of Clonality
  - Oncogene/Tumor Suppressor gene mutations
  - Tumor Specific Translocations
  - "Tissue specific" mRNA in tumor staging
  - Minimal residual disease determination

Identification Of Clonal Proliferations

- Antigen receptor gene rearrangements.
  - Southern Blotting: IgH, TCR; EBV termini.
  - PCR: Ig and TCR gene rearrangement.
- X-inactivation.
  - Human androgen receptor assay.
- Microsatellite allelotyping.



Immunoglobulin heavy chain locus - restriction enzyme digestion sites

Translocations w/o gene fusion

Translocations w/fusion product: hematologic tumors

Translocations w/chimeric products: solid tumors

Tumor	Translocation	Product
Ewing's Sarcoma	t(11;22); t(21;22); t(7;22); t(12;22)	EWS/FLI1; EWS/ERG; EWS/ETV1; EWS/ETV4
Alveolar Rhabdomyosarc.	t(1;13); t(2;13)	PAX7/FKHR; PAX3/FKHR
Synovial sarcoma	t(X;18)	SYT/SSX1
DSRCT	t(11;22)	EWS/WT1
Myxoid/round cell liposarcoma	t(12;22)	CHOP/FUS
Clear cell sarcoma soft parts	t(12;22)	EWS.ATF-1
Extraskeletal myxoid chondrosarc	t(9;22)	EWS/TEC

TRANSLOCATIONS: DETECTION METHODS

- No fusion product:
  - detect at *DNA* level.
    - In situ hybridizaton
    - Southern Blotting:
      - **Probe to translocated sequence hybridizes to altered band size. (multiple probes)**
      - **Problems: Quantity, quality of DNA, labor intensive.**
    - **PCR**
      - **If recurrent breakpoints in small DNA region**
        - **Available for BCL2 (60% detected); BCL1 (40-60% detected).**
      - **? Long-range PCR – need for high quality DNA.**

TRANSLOCATIONS: DETECTION METHODS

- Fusion product:
  - Detect at DNA *or* RNA level.
    - DNA level: FISH, Southern blotting.
    - RNA detection: RT-PCR
      - Highly sensitive.
      - Cheaper
      - "Real-time" detection.
      - Semi-quantitative detection – minimal residual disease/quantification.
      - Chimeric transcript detectable during "complete remission" : rising titer - impending relapse.
      - Need for fresh tissue (in general)

Spectral Karyotyping (SKY)

- FISH w/multiple probes to identify *all* chromosomes
  - Identify *any* translocations, markers etc. w/one test.
  - Need for special equipment
  - Need for metaphases.

Gene Amplifications & Specific Mutations

- Amplification
  - n-Myc: neuroblastoma.
  - Her2/Neu: breast cancer.
- Mutations:
  - C-Kit: gastrointestinal stromal tumors.
  - EGFR: Lung CA response to Iressa.
  - p53: poor prognosis, reduced chemosensitivity.

Tumor Suppressor Gene Mutations

"loss of function mutations"

- many possible mutations
- "hot-spots"
  - e.g., p53: Exons 6, 7, 8, 9 > 90% of mutations
- truncated protein: "protein truncation test"
- "whole gene sequencing"
  - Tumor precentage.

"Oncogene" Mutations

- :"gain of function mutations."
  - limited number for each gene.
    - "regulatory site mutations" - "constitutive activation."
    - "active site mutations" - "constitutive activation/altered substrate.
    - Often recurrent - test for known mutations.
      - e.g., c-Kit; c-RAS; *Ret*, *EGFR*, etc.

Mutations in GIST

- GIST: CD117/PDGFRA positive GI stromal tumors.
  - c-kit mutations: constitutively activated KIT tyrosine kinase.
    - Juxtamembrane domain (exon11) or transmembrane domain (exon 9)
      - Imatinib (Gleevec) responsive.
    - Tyrosine kinase domain mutations:
      - Val654Ala, Thr670Ile: Imatinib resistant

EGFR mutations in Lung CA

- 10% of patients with Lung CA – rapid response to Gefitinib (Iressa)
  - Non smokers
  - Females
  - Japanese
  - Adenocarcinoma
- Mutations in exons 18, 19, &21
  - Kinase catalytic domain
  - Increased inhibition by Gefitinib

Minimal Residual Disease

- Quantitative determination of tumor-specific fusion transcripts.
  - Presence vs quantitation.
- Detection of clone-specific sequences for T and b-cell neoplasms.
  - (Problem: ongoing mutations in antigen receptor genes).

Minimal Residual Disease/ Molecular Staging

- Cell-type specific transcript (mRNA):
  - PSA (prostate);
  - mammaglobin (breast);
  - CEA in lymph nodes (adenoCA, e.G., Colon);
  - tyrosinase (melanoma);
  - thyroglobulin (thyroid).
  - Caveat: ? cell-type specificity of low copy-number transcripts.

MRD/Molecular Staging

- RT-PCR for mets in histo negative sentinel nodes.
- Melanoma: Tyrosinase, MART-1, MAGE, GalNAc-T, PAX3
  - Variable results; ? Increased recurrence in histo-/PCR+, vs. histo-/PCR-
- Breast: Mammaglobin1, mammaglobin 2, CEA, CK19, etc.

Tumor Classification/diagnosis W/ Microarrays

- Label total RNA from a tumor
- hybridize to chip w/ ≥ 25,0000 cDNAs/oligonucleotides.
  - Expression profile unique to tumor type.
  - ? Predict behavior
  - ? Identify origin of mets
  - ? Identify targets for therapy.

Molecular Genetic Tests

- Genetic test:
  - Analyis of human
    - DNA
    - RNA
    - chromosomes
    - proteins
    - metabolites
  - to detect heritable disease-related
    - genotype,
    - phenotype
    - karyotype
  - for clinical purposes.

Genetic Diagnosis

"Purpose"

- Diagnostic Testing
- Screening
- Presymptomatic Testing
- Prenatal testing
- Preimplantation Diagnosis
- Pharmacogenetic testing
- Susceptibility to environmental agents

Genetic Alterations

- Chromosomal alterations
- "Gene-level" alterations.

Test Choice

- Cost
- Sample requirements
- Turnaround time
- Sensitivity/Specificity
- Positive/ Negative predictive value
- Type of mutation detected
- Genotyping vs mutation scanning

Conventional Cytogenetics

(Karyotyping)

- Detect numerical structural chromosomal alterations
  - trisomy
  - monosomy
  - duplications
  - translocations, etc.

Conventional Cytogenetics

(Karyotyping)

- evaluate all chromosomes
  - *prior* specification of chromosome unnecessary
  - detect unsuspected abnormality
  - detect *balanced* alterations
    - (No gain or loss of genetic material)
- FISH may be performed.
  - characterize unxpected alterations

Conventional Cytogenetics

(Karyotyping)

- Disadvantages:
  - Need for live cells to grow in culture
    - (ACMG standards, failure <1%).
  - Turnaround time - up to 10 days
    - (ACMG standards - 90% of results w/in 14 days)
  - Labor Intensive

FISH

- Use of fluorescently labeled probes to specifically visualize
  - entire chromosomes (chr. paint probes)
  - centromeres (centromeric probes)
  - specific loci (locus-specific probes)
- Metaphase
  - All types of probes
- Interphase
  - Centromeric and locus-specific probes only

FISH

- Identify:
  - translocations
  - marker chromosomes
  - Small deletions/duplications w/ locus-sopecific probes
    - e.g., DiGeorge's syndrome.

Interphase FISH

- rapid (<48 hours) detection of
  - Numerical abnormalities
  - Duplications/deletions/amplifications
  - translocations
  - mosaicism

Interphase FISH

- Prenatal Chr.13, 18, 21, X + Y
  - approx. 75-85% of all clinically relevant abnormalities.
- Dual color FISH w/ subtelomeric probes:
  - Prenatal dx of chromosomal translocations

Interphase FISH

- Need for confirmatory conventional cytogenetic testing.
- Need to specify chromosome
  - Information only about specific chromosome/locus tested.

Metaphase FISH

- Supplement conventional cytogenetics
  - Identify marker chromosomes
  - extra unknown material attached to chromosome/loss of segment
  - detect/identify rearrangements (incl. cryptic translocations),
  - identify/quantify mosaicism

Metaphase FISH

- Need to specify Chromosome/locus
  - Multiple tests to identify marker chromosome.
  - Multiprobe FISH.

Gene Dosage
Gains/Losses

- Comparative genomic hybridization (CGH)
  - Label normal and test DNA with separate dyes
  - competetively hybridize to
    - Metaphase Spread or
    - cDNA array.
  - Detect Gains and losses.

Gene Dosage
Gains/Losses

- Classical CGH
  - Hybridize to metaphase spread
    - Resolution approximately 5Mb
  - Information on *all* chromosomes
  - No need for culture.
    - can use archival material (e.g., placenta, POC, tumor etc.)
  - Single cell DNA amplification & CHG
    - applicable to preimplantation genetic diagnosis (PGD)

Gene Dosage
Gains/Losses

- Array-based CGH
  - hybridize to BAC-based or cDNA array.
  - Higher resolution (50kb vs 5MB)

Gene Dosage
Gains/Losses

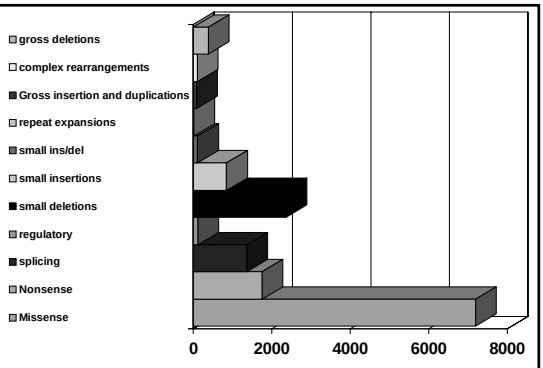
- PCR-based methods
  - Real-time (quantitative) PCR.
  - microsatellite PCR.
  - Long-range PCR.
  - probe amplification techniques.
- Rapid
- For *specific* loci
  - May be "multiplexed" for multiple loci

Molecular Tests

- Test for:
  - karyotpye
  - gain or loss of genetic material ("dosage")
  - genetic linkage
  - known/recurrent mutations
  - variations in lengths of repeat sequences
  - alterations in DNA methylation
  - unknown mutations in multiple genetic segments

Types of mutations-gene

- Point mutations
  - Missense (change an amino acid)
  - Nonsense (premature termination)
  - Silent
- Deletion
  - Large variation in size
- Insertion
- Duplication
- Splice site
- Regulatory
- Expanded repeat



Point Mutations

	ATC Leu	TTC Phe	AGC Ser	TGC Cys	GAG Glu	CTA Leu	TAT Tyr
Missense	ATC Leu	TTA Leu	AGC Ser	TGC Cys	GAG Glu	CTA Leu	TAT Tyr
Nonsense	ATC Leu	TTC Phe	AGC Ser	TGA Stop	GAG	CTA	TAT
Silent	ATC Leu	TTC Phe	AGC Ser	TGC Cys	GAG Glu	CTG Leu	TAT Tyr

Missense Mutations

- Change Amino Acid
- Change Protein Structure/function
  - Depending upon specific AA change
- Loss of function:
  - e.g., Hb S (G**A**G to G**T**G – Glu to Val), Hemochromatosis (C282Y)
- "Gain of Function":
  - e.g., Factor V Leiden
- No functional effect:
  - e.g., KVLQT1 P448R

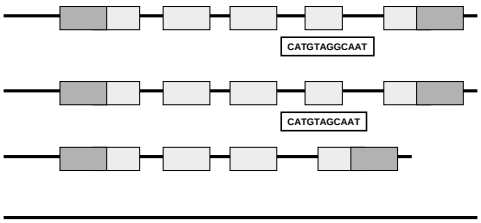
Missense mutations

- When is a missense mutation significant?
  - known structural and functional domain
  - evolutionarily conserved residue
  - independent occurrence in unrelated patients
  - absent in large control sample
  - novel appearance & cosegregation w/disease phenotype in pedigreee
  - In vitro loss of function
  - restoration of function by WT protein.

Nonsense Mutations

- Amino Acid codon to "Stop"
- Three stop codons
  - UAA, UAG, UGA
- Truncated protein
  - Protein truncation test
- E.g., Beta^0 Thalassemia in Sardinia
  - Codon 24, CAG to TAG

Deletions



Deletions

- Complete/partial gene deletion
  - Duchenne Muscular Dystrophy
  - Alpha thalassemia
- Multiple genes ("contiguous gene syndromes"
  - DiGeorge Syndrome
  - TSC2-PKD1
  - WAGR syndrome

Insertions

- Tay Sachs Disease
  - 4bp insertion in Ashkenazi Jews
- Hemophilia A
  - L1 insertion in FVIII gene (1% of patients)

Splice junction mutations

- GT/AG rule
  - AAG**GT**AAGT. .. . . //. .. . YYYYYYYYYYNC**AG**G
- Loss of splice site
  - intron not spliced out
- Creation of *novel* splice sites
  - >100 mutations
    - e.g., Hemoglobin E
    - Missense mutation and splice site error
    - Both normal and new splice site use
      - Hemoglobinopathy AND thalassemia features

Frame-shift Mutations

- Codon = 3 bp
- insertion/deletion not multiple of 3bp
  - Change of reading frame - entire protein altered.
  - e.g., Tay Sachs 4 bp insertion, BRCA1 185 delAG, BRCA2 6174delT, etc.
  - blood group O (1 bp deletion)

Other mutations

- Cap-site Mutants
- Mutations in initiation codons
- Creation of a new initiation codon
- Mutations in termination codons
- Polyadenylation/cleavage signal mutations.

Unstable trinucleotide repeats

- Fragile X Syndrome (CGG)n 5'UT
- Huntington's syndrome (CAG)n polyglutamine
- Myotonic dystrophy (CTG)n 3'UT
- SCA type 1 (CAG)n polyglutamine
- Friedrich's Ataxia (GAA)n intron 1

Mutation Testing

- Tests for recurrent mutations.
  - Limited # of specific mutations.
    - significant proportion of cases e.g., Factor V Leiden, Hemochromatosis.
- Mutation Scanning Methods.
  - Multiple "private" mutations of one or more genes.
    - e.g., BMPR2 mutations in familial PPH,
- Combination.
  - e.g., BRCA1/2, CFTR etc.

Recurrent Mutation Tests

- Many rapid methods.
- High sensitivity/specificity.
- Test choice - laboratory preference
  - Workflow, equipment, kit availability
  - patent issues, etc.
- Detect
  - heterozygotes,
  - compound heterozygotes
  - homozygotes

Recurrent Mutation Tests

- Choice of mutation tested
  - Clinical syndrome
  - Family history
  - Ethnicity
- Positive results
  - Unambiguous
  - Technical false positive rare (*most* methods)
  - Positive predictive value, penetrance, etc. usu known

Recurrent Mutation Tests

- Negative predictive value:
  - Population screening:
    - 1- (ethnic prevalence x [1 - sensitivity for specific ethnic group])
  - Family history (index case w/ unknown mut)
    - 1 - (prior probability x [1- sensitivity for specific ethnic group])
  - Family history (known mutation in index case)
    - 100%
  - Affected individual (unknown mutation)
    - 0%

Recurrent Mutations

- Methods
  - PCR-RFLP
  - Allele-specific probes/primers
  - Direct sequencing/"Minisequencing"/ Pyrosequencing.
  - Molecular Beacons/TaqMan probes.
  - Oligonucleotide ligation assay.
  - Mass spectroscopy-based methods.

PCR-RFLP

- Quick, Robust
- Sources of error:
  - Amplification failure of one allele
    - linkage disequilibrium with primer site polymorphism (HFE)
  - Failure of restriction enzyme
    - Control in same tube
  - Different variants with loss of same restriction site

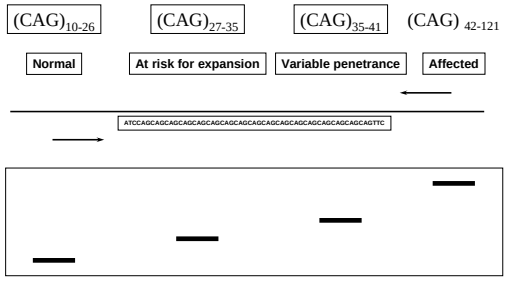
Real-Time PCR

- New instruments can monitor PCR during thermocycling
  - intercalating dye:
    - non-specific increase in fluorescence with increased double-stranded DNA
    - "Melting curve" analysis - monitor denaturation of double-stranded DNA
  - Probes using Fluorescence Resonance Energy Transfer ("FRET")
    - Monitor binding of probe to wild-type or mutatnt allele.

Repeat Expansions

- Southern Blotting Methods
  - Gold Standard
  - Labor intensive
  - need for high quality DNA
- PCR-based Methods
  - Rapid
  - Amplification failure with very long repeats.

Expanded Repeats-Huntington Disease



Mutation Scanning Methods

- Test one or more genes for unknown variation in.
  - Exons
  - Introns
  - splice sites
  - Promoters/enhancers
  - "locus control region"

Mutation Scanning Methods

- Ideal method:
  - Screen large DNA sequence
  - 100% sensitivity and specificity
  - Unambiguously define mutation.
  - Minimum # of steps
  - High throughput
  - No special equipment
  - No dangerous reagents
- No such method
  - Compromise

Screening Methods

- physical properties of amplified gene segments
  - denaturation profile, electrophoretic mobility, etc.
    - SSCP
    - DGGE
    - DHPLC
    - Cleavase fragment length polymorphisms
    - heteroduplex analysis
    - dideoxy fingerprinting.

Screening methods

- Sensitivity determined by specific mutation
- Need for multiple conditions
- *One* datapoint per gene segment evaluated
- Screen for *presence,* not *identitiy* of mutation.

Mutation Scanning Methods

- Direct Sequencing
  - Screen presence *and* identity of mutation
  - Bidirectional sequencing
  - 2 data-points *per base* sequenced.
  - DNA sequencing
    - usu. multiple exons tested.
    - splice-site mutations may be missed, especially mutations deep in large introns.
  - RNA sequencing
    - need for cells w/c express gene
    - "nonsense mediated decay"
    - RNA more labile

Direct Sequencing Methods

- Automated fluorescent sequencing
  - DNA/cDNA amplification, purification, and re-amplification with Fluorescent "Big-Dye" terminators.
  - widely available
  - need to visually scan electropherograms
    - verify "base calling", heterozygous bases

Direct Sequencing Methods

- Pyrosequencing
  - limited to short seqences.
  - need to optimize algorithm for each segment
- Chip-based" sequencing
  - rapid
  - reduced sensitivity for heterozygous and frame-shift mutations.

Interpretation of Variant

- Previously reported variant
  - Known to be cause of disorder
  - Known to be "neutral variation"

Interpretation of Variant

- New variant:
  - Type likely to be assoc. w/disorder
    - frame-shift mutation
    - start "ATG" mutation
    - "Stop codon"
    - splice-junction mutation
    - non-conservative missense in active site,

Interpretation of Variant

- New Variant
  - Type likely to be "neutral"
    - e.g., no change in amino acid, and not cryptic splice site
  - Type w/c may or may not be assoc. w/ disorder
    - E.g., non-conservative missense mutation, in region not known to be active site, etc

Interpretation of Variant

- Recessive Disorders.
  - Test parents to ensure two variants in *trans* (separate alleles) not in *cis (*same allele).

Testing Strategies.

- Single gene disease w/ only recurrent mutations (e.g. Achondroplasia or MEN2)
  - Test for recurrent mutation
  - Positive result
    - penetrance known
  - Negative result
    - False negative rate known.
  - Phenotypic testing, if indicated.

Testing Strategies.

- Single gene ds w/recurrent and private mutations (e.g., CFTR, thalassemias).
  - test for "ethnic" recurrent mutation(s)
  - If positive, singificance known
  - If negative, and index case or relative, perform "mutation scanning" test.
    - if positive, probably significant, family testing may help.
    - if negative, significance depends on whether index case or relative.

Testing Strategies.

- "Single gene" condition w/ repeat polymorphisms (Fragile X)
  - Test for repeat polymorphisms using either
    - Southern Blotting
    - PCR (very large expansions may be missed)
- Clinical sydrome w/ multiple genes
  - "recurrent" (SCA)
  - Private (Long QT)

Testing Strategies

Cystic Fibrosis

CFTR Screening

- Carrier frequency in various ethnic populations
  - European Caucasian: 1/25
  - Ashkenazi Jewish 1/25
  - Hispanic American 1/46
  - African American 1/65
  - Asian American 1/90

CFTR Screening

- CFTR Gene:
  - 250 kb
  - 27 Exons
  - 6.5kb mRNA
  - In-frame deletion of codon 508 in 70% of cases (Caucasians/Ashkenazim)
  - >1000 mutations reported

CFTR Screening

- "…recommended that testing for gene mutations that cause cystic fibrosis be offered as an option to all pregnant couples and those planning pregnancy."

CFTR Screening

- ACMG recommendations
  - Testing offered to all Caucasians and Ashkenazim, made available to all other ethnic groups
  - Simultaneous or sequential couple screening
    - Give results to both partners

CFTR Screening

Universal pan-ethnic core mutation panel consisting of:

- 25 mutations.
- 3 exonic polymorphisms as reflex tests.
- 5/7/9T intronic polymorphism as reflex test only if R117H is positive.

CFTR Screening

- Extended mutation panels for positive-negative couples not encouraged
- Reporting of results and residual risks should be based on model report forms developed by ACMG committee
- Primary care providers uncomfortable w/ these complexities should refer pt to genetic counselor

CFTR Screening

- 5T/7T/9T intronic polymphorphism
- R117H + 5T in *cis* - CF
- R117H + 7T in *cis* - CBAVD
- R117H (etc.) + 5T in *trans* – CBAVD
- 5T/5T homozygosity - CBAVD
  - R117H causes CF only when w/ 5T on same allele
  - 5T with least efficiency of RNA processing
  - 5T in 5% of US population

CFTR Screening

- Limitations
  - Inability to detect all CF mutations
  - Correct paternity assumed; results applicable only for current reproductive partners
  - Assumes family history is truly negative
  - Poor genotype-phenotype correlation - prognostic prediction in affected offspring difficult

CFTR Screening

- Concurrent testing: Both partners screened, both informed.
- Advantages:
  - Quicker
  - Alerts both partners
    - w/ current and future partners
  - Informs both families of potential risk
- Disadvantage:
  - Anxiety
  - Cost

CFTR carrier Screening

- Sequential
- Advantages:
  - More efficient when low carrier rate
  - Less potential anxiety
- Disadvantages
  - Higher residual risk
  - No information for family of partner not tested

CFTR: INCIDENCE, CARRIER, MUTATION RATES: BY POPULATION

Group	Incidence	Carrier freq.	%ΔF508	% other "common"	% group-specific	Sensitivity
Caucasian	1:3,300	1/29	70	13		80-90%
Hispanic	1/8-9000	1/46	46	11		57%
Ashkenazim	1:3,300	1/29	30	67		97%
Native Am.	1:1500 – 3970		0	25	69	94%
African Am.	1:15,300	1:60-65	48	4	23	75%
Asian Am.	1:32,100	1:90	30	0	0	30%

Negative results: + family history

- Caucasian Couple
- each w/ sibling with CF.
  - (Prior Probability of each parent being a carrier = 2/3).
- Both test negative for the 25 mutations.
  - Probability parent is carrier = (0.67 *(1-.90)) = 0.067.
  - Probability *both* parents carriers = .004489
  - Probability of affected child = 1 in 900

Negative Results: + Family History

- Hispanic Couple w/ same history and results:
  - Probability of being carrier = (0.67 *(1-0.57)) = 0.287;
  - probability of an affected child = 1 in 48!
  - (versus untested prob.: .67*.67*.25=1/9)
- Asian couple w/ same hx and results:
  - probability of affected child 1 in 18!

Genetic testing additional considerations:

- Benefits Vs. Risk of Testing:
  - Availability of treatment/prevention of clinical syndrome
  - Presence or absence of pre-clinical manifestations.
  - Discrimination:
    - Insurance
    - Employment
    - Confidentiality

Additional Considerations

- Screening vs testing "index" case.
- Index case.
  - Known disease;
  - negative result:
    - mutation not detected
    - carrier testing not possible.
- Locus heterogeniety:
  - Long QT; red-cell membrane defects; phenylketonuria; etc.
- Variable "penetrance"
  - variable predictive value of positive results
- Variable expressivity

Additional Considerations

- Potential interventions:
  - Behavioral
    - lung cancer-risk - smoking cessation;
    - heart disease risk - diet/exercise;
    - risk of breast/colon cancer - screening accepatnce.
  - Medical
    - e.g., prophylactic mastectomy/thyroidectomy;
    - blood-letting/blood donation for HFE;
    - anti-arrhythmics for Long QT, etc.

Additional Considerations

- Pre-morbid/clinical syndrome
  - Is there a clinically identifiable syndrome ?
  - ? Need for intervention *prior* to clinical manifestations
- Technical considerations
  - e.g., Fragile X-syndrome.
- Patent issues
  - affect availability/cost of testing

Additional Considerations

- Ethics
  - implications for patients *and* relatives.
    - e.g., identical twins; siblings;
    - paternity issues -
- Legal issues
  - New York State Civil Right Law:
    - Need for nformed consent
      - Genetic testing only (not phenotypic testing)
      - Standards for informed consent in civil rights law, section 79-l [http://assembly.state.ny.us/leg/?cl=17&a=12].

Factors affecting utility of genetic testing

- **Increased Utility**
  - **High morbidity and mortality of the disease**
  - **Effective but imperfect treatment**
  - **High predictive power of genetic test (high penetrance)**
  - **High cost or onerous nature of screening and surveillance methods**
  - **Preventive measures that are expensive or associated with adverse effects**

Modified from: BMJ: 322: 1054; April 28, 2001.

- **Decreased utility**
  - **Low morbidity and mortality of disease**
  - **Highly effective and acceptable treatment (i.e., no harm is done by waiting for clinical disease to treat patient)**
  - **Poor predictive power of the genetic test (low penetrance)**
  - **Availability of inexpensive, acceptable, and effective surveillance methods (or need for surveillance whether or not one has increased genetic risk)**
  - **Preventive measures that are inexpensive, efficacious, and highly acceptable - e.g., folate supplementation.**

Ordering Molecular Tests

- Patient preparation: None
  - **Avoid heparin:** interferes with PCR.
- Specimens:
  - Fresh whole blood: EDTA/Citrate
  - Fresh tissues
  - Frozen tissues
  - Paraffin embeded tissues
  - Slides etc.

Ordering Molecular Tests

- Specimen Handling
- DNA-based tests:
  - Room temperature, up to 72 hours (maybe more with special buffers)
- RNA-based tests:
  - Deliver ASAP (4-6 hours)
  - Special considerations for proprietary test.

Ordering Molecular Tests

- Essential info (Molecular Genetic Tests):
  - Clinical information
  - pedigree, if possible
  - Race
  - reason for testing.
- Informed consent:
  - New York State Civil Rights Law.
    - Nature of test; availability of genetic counseling; implications of positive and negative tests, etc.