THE FUTURE IS GENOMIC MEDICINE

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What is “Personalized Medicine”?
Personalized Medicine

= Molecular Medicine

What is “Molecular Medicine”?

Molecular Medicine

- Gene-level diagnostics
- Gene-level therapeutics
Is Whole-Genome Sequencing the Ultimate “Personalized Medicine”? 

THE HUMAN GENOME PROJECT Timeline

1985 Exploratory conferences held at UC-Santa Cruz and Santa Fe 
1986 Human Genome Initiative announced by DOE 
1987 NIH funding commences; 15-year plan formulated 
1988 HUGO founded 
1989 ELSI established 
1990 15-year NIH-DOE project formally begins; $3 billion in funding pledged 
1991 Genome Database established 
1992 Low-resolution linkage map of entire human genome published 
1993 First 5-year plan revised 
1994 First 5-year goal achieved one year ahead of schedule 
1995 High-resolution physical maps of chromosomes 16 and 19 completed 
1996 Yeast genome sequence completed 
1997 Human genome physical map with 30,000 STS’s achieved 
1998 Task Force on Genetic Testing releases report 
1999 First human chromosome (#22) completely sequenced 

Sanger Sequencing
Next-Generation DNA Sequencing

Advancing genetic analysis
one billion bases at a time
Molecular Medicine
• One gene/one disease

Genomic Medicine
• *All* genes/*all* diseases
Array Comparative Genomic Hybridization (aCGH)
Chromosomal Microarray (CMA)
Whole Genome Data Is Acquired

- Patient below without any known genetic disease
- All chromosomes but Y represented

Xp21 Complex Glycerol Kinase Deficiency

- 6.66 Mb deletion
- X chromosome

ACMG Recommends Replacing Karyotyping with Chromosomal Microarrays as 'First-Line' Postnatal Test

Microarrays should be used instead of G-banded karyotyping as the first test to detect genetic abnormalities in postnatal evaluations, according to the American College of Medical Genetics.
CNVs are common in all genomes surveyed...

And sequence variants are even more common...

Incidentalome
A Little Taste of the Challenge Ahead: Sequencing Experience With BRCA1 & 2

- Complete sequencing of both genes in >150,000 people at Myriad Genetics alone
- >10,000 mutations and benign or uncertain variants identified

[B. Ward, personal communication]
ACMG RECOMMENDED CORE MUTATION PANEL FOR
GENERAL POPULATION OF CARRIER SCREENING

ΔF508  Δ507  G551D  W1282X  N1303K
R553X  621+1G>T  R117H  1717-1G>A  A455E
R1162X  G85E  R334W  R347P  711+1G>T
3120+1G>A  2184delA  1078delT  3849+10kbC>T
3659delC  1898+1G>A  750+5G>A  1148T

Classes of Novel/Unexpected
Sequence Variants Identified by
Whole Genome/Exome Sequencing

• Missense variants of uncertain significance
  in known gene
• Variants and deleterious mutations in
  unknown gene(s)
• Deleterious mutations in unintended target
  (e.g., BRCA mutations in a baby)
WGS Represents a Sea-Change in Clinical Laboratory Testing:

For the first time, patients will need to choose beforehand what portions of the test results they wish to receive or not receive.

Informed Consent for Whole Genome/Exome Sequencing: Patient Choices

- Receive all information (CD, DVD?)
- Receive relevant/targeted information
- Receive medically actionable information for patient’s age
- Receive medically actionable information for future
- Receive medically actionable information for relatives

Disease Gene Panels for Next-Generation Sequencing

- Hypertrophic cardiomyopathy
- Dilated cardiomyopathy
- Hereditary arrhythmias (channelopathies)
- Retinitis pigmentosa
- Albinism
- Mental retardation
- DNA repair defects
- Skeletal dysplasias
- Disorders of sexual development
- Hearing loss
Whole-Exome Sequencing of Germline DNA

Disease Gene Discovery by Whole-Exome Sequencing

Kabuki Syndrome
EVOLUTION OF MOLECULAR GENETIC TESTING

- Single gene/Single mutation
- Single gene/Multiple mutation panel
- Multiple genes/Multiple mutations
- Whole-gene sequencing
- Multiple whole-gene sequencing panel
- Whole-exome sequencing
- Whole-genome sequencing

Ethical Dilemmas of Whole Genome Sequencing

- Revelation of "off-target" mutations
- Many revealed disorders will have no prevention or treatment
- Revelation of nonpaternity, consanguinity, incest
- Costs of genetic counseling and follow-up
- Possible forensic uses of data
- Data storage and privacy
- Huge number of novel missense variants

Clinical Genomics Board

- Molecular/genomic laboratory directors
- Laboratory technical staff
- Genomic informaticists
- Clinical geneticists
- Genetic counselors
- Residents/fellows
- Ordering clinicians
RESULTS:
One homozygous, probable disease causing variant E359X in AAAS was detected. Other observed variants, within the primary gene list, are not convincingly demonstrated to be causal.

INTERPRETATION:
The data are consistent with a 46, XX female. There are 10 intervals greater than 5 Mb that are homozygous-by-descent, suggesting consanguinity. There is no evidence of any copy number abnormalities (gain or loss) involving an entire chromosome arm.

The following primary gene list associated with the keyword(s) below was generated using the HGMD (Human Gene Mutation Database) Professional Version 2011.3:

Primary Gene List: ALAD, ALS2, ANG, APEX1, CHD3, CHGB, CHMP2B, CRYM, DAO, DEFN1, FER, FEN1, GEN, LUM, OFDM1, EGR1, PKD1, FOS, FOS, FOSB, HSPA1A, IGF2, IPK1, JNK1, KIF1A, KIF1B, KIF20A, LAMA1, LEF1, LRP5, MAFA, MAP2K1, MAP2K2, MAP2K3, NDUFB10, NFKB1, OAS1, OAS2, OAS3, PDGFA, PDGFB, PGR, PRPS1, PRPS2, PRPF3, PRPF8, PRPF19, PRPF43, PRPF80, PRPF46, PRPF81, PSEN1, PSEN2, SNTA1, TNFRSF1A, TNFRSF1B, TNFRSF12A, TNFRSF12B, TNFRSF18, VAPB, VBP1, VCP, ZBTB32, ZC3H12A

Keywords: Amyotrophic lateral sclerosis, ALS

Protein changing variants within the homozygous intervals: Within the homozygous intervals, the following four probable disease-causing variants were found. Of these, a nonsense mutation expected to cause protein truncation in gene AAAS seems to correlate with patient’s clinical symptoms. Defects in this gene are reported to cause achalasia-addisonianism-alacrima syndrome (AAAS or ‘Triple A syndrome’) (Ref 2).

RESULTS:
This patient has a strong family history of presumed prion disease, best fitting into the fatal familial insomnia category. A heterozygous mutation E200K in PRNP, which is well documented to cause Creutzfeldt-Jakob disease (CJD) [MIM:123400], was identified. Other variants affecting the protein coding sequence of the primary gene list (below) were identified but are of unknown significance.

INTERPRETATION:
The data are consistent with a 46, XY male. There is no evidence of consanguinity or any copy number abnormalities (gain or loss) involving an entire chromosome arm.

The following primary gene list was generated based on known association with the diagnoses provided by the ordering physician and was generated using the HGMD (Human Gene Mutation Database) Professional Version 2011.3:

Primary Gene List: PRNP, CACNA1A, PNP, C3, ADA, GABRB3, MIR182

Keywords: vasculitis, prion, fatal familial insomnia, familial insomnia, insomnia, CJD, Creutzfeld-Jacob, Creutzfeld Jacob, cognitive decline

Preliminary Communication

PRENATAL SEX DETERMINATION BY DNA AMPLIFICATION FROM MATERNAL PERIPHERAL BLOOD

Y.-M. D. Lo¹, S. Patel¹, J. S. Warnock¹, M. D. G. Gilmore³, S. Sampietro¹

University of Oxford Clinical School, Department of Haematology, Maternity Department, ¹ and Nuffield Department of Orthopaedics, John Radcliffe Hospital, Oxford, ² and A. Benciolli

Bueno Hemophilia and Thrombosis Centre, University of Milan, Italy

Summary: The polymerase chain reaction was used to amplify a Y-specific repeat sequence in peripheral blood DNA samples from 19 pregnant women who had a gestational age of 9 to 46 weeks. Y-specific sequences were amplified from all 12 women who bore a male fetus but in none of 7 women who bore a female fetus. With stringent precautions against contamination, this technique may assist prenatal diagnosis of sex-linked genetic disorders.
Rare Male Fetal Cell in Maternal Blood

Relative Proportion of Sequence Reads Per Chromosome by Massively Parallel Sequencing

Z-scores for Chromosome-21 Sequence in Affected and Unaffected Pregnancies

Chiu et al., PNAS, 2008

Chiu et al., BMJ, 2011
Some Ethical Issues Raised by Noninvasive Prenatal Diagnosis

• Uncertain informed consent requirements
• Uncertain pre-test genetic counseling requirements
• Detection of “off-target” disorders
• Ease of specimen collection allows DTC testing
• Ease of specimen collection prompts screening for minor disorders, traits, sex, paternity, adult-onset disorders
• Potential for “trivialization” of prenatal diagnosis and pregnancy termination
Should whole-genome/exome sequencing be applied to:

- Newborn screening?
- Couple screening?
- Population screening?
- Employment screening?

NIH Task Force on Genetic Testing

Testing of Children

Genetic testing of children for adult onset diseases should not be undertaken unless direct medical benefit will accrue to the child and this benefit would be lost by waiting until the child has reached adulthood. The Task Force agrees with the American Society of Human Genetics and the American College of Medical Genetics that “Timely medical benefit to the child should be the primary justification for genetic testing in children and adolescents.” Although

GINA

What’s in your genes?

SAVING
GENETIC
DISCRIMINATION

GINA.org
NGS Service Delivery

- **Who will run it:** analyze and interpret in-house, or receive raw data from outside sequencing lab?
- **Who will lead it:** Geneticists for germline mutations, pathologists for somatic mutations (tumors)?
- **Who will understand it:** Need for new content in training programs and board exams — “medical genencist” or “genomic pathologist”?
- **Who will pay for it:** Wealthy patients out of pocket? Will insurers consider it “investigational”?
- **Need for professional practice guidelines**
- **Will parts of the genome be off-limits?**

A Sample of Genetic Testing Patents

- 5,753,441 BRCA1
- 5,753,438 Hereditary hemochromatosis
- 5,693,470 Non-polyposis colorectal cancer
- 5,686,240 Niemann-Pick disease
- 5,661,419 Ulcer, colitis and Crohn’s disease
- 5,675,255 Canavan disease
- 5,676,350 Pseudomyotonia congenita
- 5,654,725 Hereditary breast cancer
- 5,651,588 Von Hippel-Lindau (VHL)
- 5,650,282 Williams syndrome
- 5,645,993 Breast or ovarian cancer
- 5,639,614 Leber’s optic neuropathy
- 5,639,607 Lead sensitivity

Impact of Gene Patents on Healthcare

- Limited access and noncompetitive pricing
- Increased healthcare costs
- Lack of peer review and comparison
- Hampered quality assurance
- Potential undetected systematic errors
- Interference with medical training
- Restricted opportunity and incentive for test improvements and advancement of the field
- **Missing or masked targets on microarrays and whole-genome sequencing?**
Key Plaintiffs in the ACLU Suit

- Association for Molecular Pathology
- American College of Medical Genetics
- American Society for Clinical Pathology
- College of American Pathologists
- Academic geneticists whose *BRCA* testing was shut down
- Breast Cancer Action Network
- Individual breast cancer patients

Key Arguments in the ACLU Suit

- Genes are products of nature, not inventions.
- It is unconstitutional to patent a person’s individuality.
- Patients are prevented from seeking a “second opinion”.
- Gene patents are overly broad.
- Legal principles bar patenting of laws of nature, products of nature, and abstract ideas.
- Gene patents violate the First Amendment by inhibiting free speech and access to information.
Key Arguments of the Defendants

• The 7 patents deal with “isolated” BRCA genes.
• “These isolated molecules are man-made chemical compositions, structurally and functionally distinct from any substance found in the human body – indeed, in all of nature.”
• The method claims involve unique molecular tools such as DNA probes and primers.
• The inventions made familial breast/ovarian cancer testing practical.
• ‘Plaintiffs’ case is nominally directed to Myriad, but actually imperils the entire biotechnology industry – molecular diagnostics, therapeutic drugs, agricultural applications, animal husbandry, etc.”
• There is no evidence that Myriad has exerted any “adverse legal interest” or damages on the plaintiffs.

Progress of the ACLU Suit

• Filed May 2009 in New York Southern District Federal Court
• Immediate Move to Dismiss rejected
• Judge Robert Sweet issues Intention to Hear the Case, November 2009
• Judge Sweet issues Ruling, March 29, 2010
• Myriad appeals the decision to Court of Appeals for the Federal Circuit
• Three-judge panel rules 2-to-1 in favor of defendants
• Plaintiffs appeal case to the U.S. Supreme Court
• Supreme Court remands case back to CAFC
• CAFC again rules 2-to-1 in favor of defendants
• Plaintiffs re-appeal case back to U.S. Supreme Court
“Next-Next” Generation Sequencing