Cases in Genetic Testing in Primary Care

January, 2023

RYAN M. HARDEN, MD MS
PHYSICIAN, GATEWAY FAMILY HEALTH CLINIC
ASSISTANT PROFESSOR, UNIVERSITY OF MINNESOTA MEDICAL SCHOOL, DULUTH CAMPUS
INSTRUCTOR, KAPLAN MEDICAL

1

Disclosures

- I have no pertinent financial relationships to disclose.

2

Objectives

- Define genetic testing
- Discuss history, implementation and future of genetic medicine
- Review relevant genetic concepts
- Discuss examples of genetic testing already implemented in clinical medicine
- Discuss how some tests are performed
- Explore the role of the primary care physician in genetic testing
- Touch on some of the ethics of genetics testing

3

What IS genetic testing?

- Genetic testing is a medical test that identifies changes in chromosomes, genes or proteins.
  - Molecular
  - Chromosomal
  - Biochemical

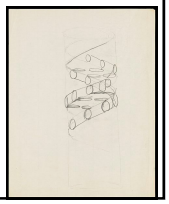
4

What IS genetic testing?

- Central Dogma of Molecular Biology:

  DNA → RNA → Protein

- Can confirm or rule out a genetic condition or determine chance of developing or passing on a genetic disorder
- More than 1,000 Genetic tests are currently in use



5

Etiology of genetic disease

- Central Dogma of Molecular Biology:

  DNA → RNA → Protein

- Protein may be less (or more) active due to mutation
- Protein may be over (or under) expressed due to mutation
- Protein may fold up inappropriately in three dimensions, affecting function
- Protein may be shorter (or longer) than usual, affecting function
- Protein may not end up in the correct place for normal function

6

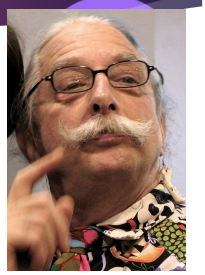
Why perform genetic testing?

- Down Syndrome (1/600)
- Cystic Fibrosis (1/2500 Caucasian Americans)
- Fragile X Syndrome (1/800 to 1/1000)
- Sickle Cell Anemia (1/500 people with ancestry in malaria-endemic areas)
- Hemochromatosis (1/450)
- Breast Cancer (1/8 women)
- Sex Chromosome Aneuploidy (1/1000)
- Congenital Heart Disease (1/100)

7

52 yo M

- Patient: "Doc, I want you to do blood tests on me."
- Me: "Which tests do you want me to do?"
- Patient: "All of them."



Patch Adams

8

Newborn Metabolic Screening

- Public Health Program
- Screening all infants
- Testing for metabolites or enzyme activity
- Common diseases which are treatable and difficult to diagnose early
- Example: Phenylketonuria
  - Inability to metabolize phenylalanine
  - Incidence: 1/15,000
  - If treated early (diet) can limit long-term damage

9

NEWBORN METABOLIC SCREENING

Results	
Amino Acidemia (Includes PKU) *	Negative
Biotinidase Deficiency	Negative
Congenital Adrenal Hyperplasia (CAH)	Negative
Congenital Hypothyroidism	Negative
Cystic Fibrosis	Negative
Fatty Acid Oxidation *	Negative
Galactosemia	Negative
Hemoglobinopathy (Includes Sickle Cell Disease)	Normal
Organic Acidemia *	Negative

Now that we all agree we are already DOING genetic testing…….

10

Case # 1 (2011)

- 40 yo F new to practice to establish care for chronic pain
- Chart notes mother died of breast cancer in 40's; further questioning reveals 19 yo niece just died of breast cancer
- She has never had a mammogram and last clinical breast exam was 2 years ago

11

Breast Cancer

- Strong genetic component
- 3x increased risk if one first degree relative affected; 10x if two first-degree relatives affected
- BRCA1 (17q21) mutation cause ½ of genetic breast cancer (prevalence 1/500 to 1/1000)
- BRCA2 (13q12.3) mutations cause 1/3 of genetic breast cancer (prevalence 1/250 to 1/500)
- Collectively less than 5% of breast cancer in the population

12

BRCA mutations

- ***BRCA1*** and ***BRCA2*** are **TUMOR SUPPRESSOR GENES**
  - the protein product of these genes is involved in regulating DNA repair
- If you INHERIT ONE MUTATION, you need to ACQUIRE ANOTHER MUTATION
  - **Two Hit Hypothesis**: loss of heterozygosity; hypermethylation (gene silencing)
- Hundreds of mutations have been identified in the BRCA genes; some are harmful some are not
- This is a demonstration of the concept of **allelic heterogeneity**
  - **FOUNDER EFFECT**: Iceland: BRCA2 999del5 mutation
- Identification of mutations involves DNA SEQUENCING

13

May 27, 2013

At age 37, Angelina Jolie underwent
Preventive bilateral mastectomy
After finding out her lifetime risk of
Developing breast cancer was 87%
Due to BRCA1 mutation

As a result of this publicity,
Though testing INCREASED, percentage
Of abnormal results stayed the same

14

BRCA "Degree of Penetrance"

- BRCA1 or BRCA2 carriers from families with strong history of breast cancer have >80% chance of breast cancer by age 70
- BRCA1 or BRCA2 carriers from families without strong history of breast cancer have 45% to 60% chance of breast cancer by age 70

15

Case # 1

- Patient saw Genetic Counselor
- Initially opted to NOT have BRCA testing done as not covered by insurance
- Ultimately had BRCA testing and tested positive
- Lifetime risk of Breast Cancer 80+%
- Opted for Prophylactic Bilateral Mastectomy
- Decreased lifetime risk to 5%

16

57 yo F to establish local care due to cancer diagnosis

- Managed at Mayo
- BRCA- positive
- Opted to not have mastectomy ("All my family with breast cancer got it in their 40's!)
- S/P bilateral mastectomy
- Chemotherapy

17

BRCA testing

- Previously done at Myriad Genetics
- In 1996, BRCA testing was $3,340 for comprehensive test
- $500 M annual revenue in 2012
- Association for Molecular Biology vs. Myriad Genetics lawsuit in 2013
- US SUPREME COURT: A naturally occurring DNA segment is a product of nature and not patent eligible merely because it has been isolated.

18

BRCA testing in Primary Care

- The USPSTF recommends that **primary care clinicians** assess women with a personal or family history of breast, ovarian, tubal, or peritoneal cancer or who have an **ancestry associated with breast cancer susceptibility 1 and 2 (BRCA1/2) gene mutations** with an appropriate brief familial risk assessment tool. Women with a positive result on the risk assessment tool should receive **genetic counseling** and, if indicated after counseling, genetic testing. ***GRADE B RECOMMENDATION***

19

Beneficence

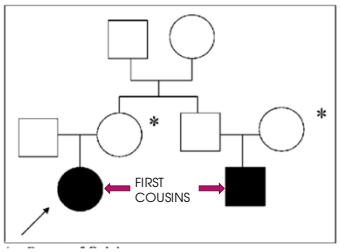
- It is important for the primary care provider to understand the criteria for testing for BRCA gene defects

20

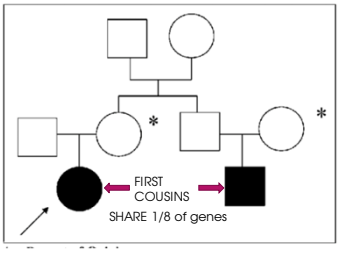
55 yo M with first cousin with CHEK2 mutation

- CHEK2 gene codes for protein that STOPS TUMOR GROWTH
- Mutation is CHEK2 doubles risk for breast cancer
- Also associated with colon cancer, prostate cancer, stomach cancer and kidney and sarcoma

21



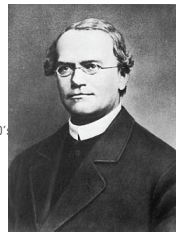
22



23

A brief History of Genetics

- Mendel's pea flower garden: 1864
- Structure of DNA Elucidated: 1952
- Identification of clinical chromosome disorders 1959
- Gene Sequencing and Cloning: 1970's
- Gene Mapping: 1980's
- Identification of genetic disease-based mutations 1990's
- Human Genome Project completed: 2001



24

Why perform genetic testing?

- Disease diagnosis
- Carrier detection
- Risk Assessment

25

How is genetic testing performed?

- Office
- Lab
- Direct to Consumer

26

Types of Genetic Testing

- Predictive
- Prenatal
- Diagnostic
- Single gene testing
- Multigene panels
- Genotyping
- Genome sequencing
- Microarrays
- Cytogenetic testing

27

Clinical Validity

- Testing positive for a gene doesn't always mean you'll get the disease
  - But sometimes it does
- Testing negative for a gene doesn't always mean you won't get the disease
  - But sometimes it does
- Penetrance: Likelihood you will get the disease "phenotype" if you get the disease "genotype"

28

Genetic Counseling

- Medical Professionals that have the expertise to
  - Help patients understand implications of positive or negative test for self and family
  - Help patients obtain insurance coverage for tests
  - Discuss issues related to inheritance and family planning
  - Provide patient with informed consent

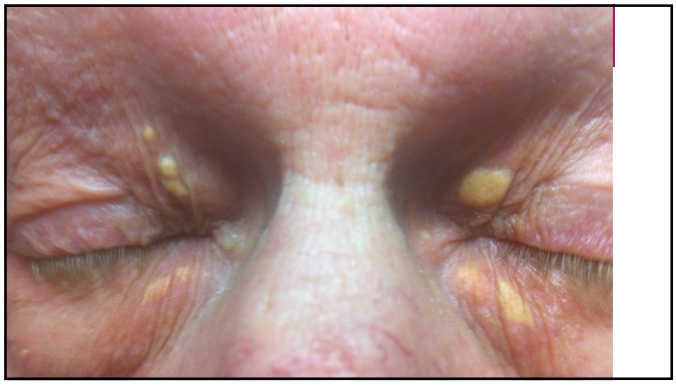
29

62 yo M

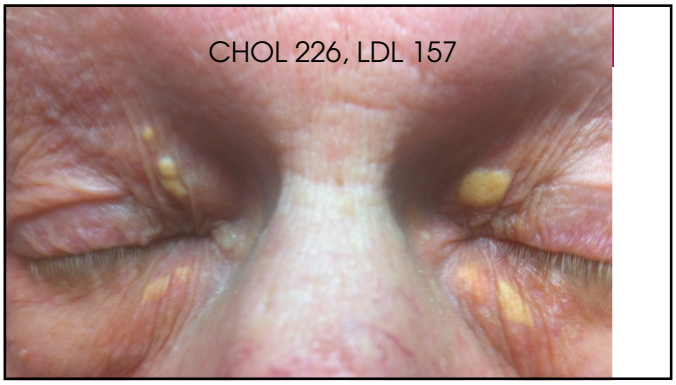
- Establish care with new provider for chronic back pain. He gets regular injections for chronic low back pain and needs an order for that
- His only medications are omeprazole and hydrocodone (6 tablets per week)
- He admits "I don't really like doctors" when you ask him when his last complete physical was.

On exam, you notice.....

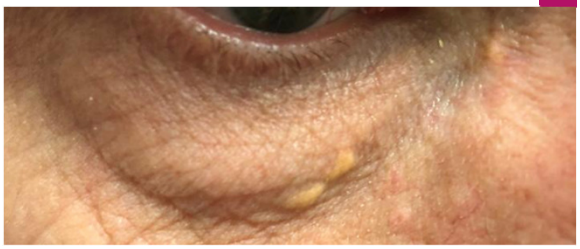
30



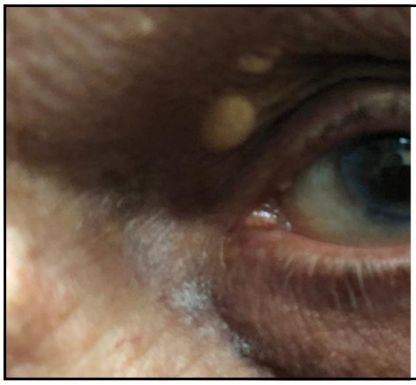
31



32



33



Xanthoma:
Xanth = "yellow"
-oma = space occupying Lesion

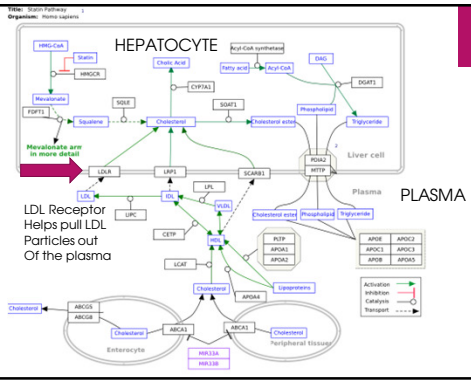
Associated with hyperlipidemia

34

Hyperlipidemia

- Familial Hypercholesterolemia is an **autosomal dominant** disorder of lipid metabolism caused by mutations in LDL-Receptor
- **Loss of Function Mutation**
- 66-80% of LDL cleared from bloodstream
- **Allelic Heterogeneity**: caused by insertions, deletions. VARIABLE EXPRESSION
- Prevalence **1/500**
- Generally, Untreated cholesterol levels are >300mg/dL
- Most hyperlipidemias due to MULTIFACTORIAL INHERITANCE pattern/environment
- May be associated with **xanthomas**
- Results in premature atherosclerosis

35



36

Screening for Familial hypercholesterolemia

- Familial hypercholesterolemia
  - Group of genetic disorders that result in elevation of plasma lipids; premature atherosclerosis
  - One of the more common conditions is mutation in gene encoding LDL receptor
- Fasting lipid profile
- Routine lab test
- Treatment: statin therapy

37

Familial Hypercholesterolemia

- **ENVIRONMENT** plays a major role
- Some populations with heterozygosity of LDL receptor defects have normal rates of atherosclerosis
- Some individuals with two NORMAL copies of LDL receptor develop atherosclerosis
- ACC recommends if LDL >190 age 40-75, maximum tolerated statin
- Current recommendations are to treat LDL>190 at 10 years of age if no family history of premature CAD, but treat at LDL >160 if family history of premature CAD

38

Current Recommendations

- The USPSTF recommends screening **women aged 20-45 for lipid disorders if they are at increased risk for coronary heart disease**. The USPSTF recommends screening men aged 20-35 for lipid disorders if they are at increased risk for coronary heart disease.
- The American Academy of Pediatrics (AAP) recommends **all children between 9 and 11 years old are screened for high blood cholesterol levels** due to the growing epidemic of obesity in children. For children in these categories, their first cholesterol test should be after 2 years but no later than 10 years of age.
- The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for lipid disorders in children and adolescents 20 years or younger. ***Grade I recommendation***

39

Familial Hypercholesterolemia

- There is NO BENEFIT to performing genetic testing to identify patients with FH
- Follow lipid screening guidelines, treat as per recommendations

40

Marfan's Syndrome

- A 27 year old medical student notices they have some of the physical findings consistent with Marfan's Syndrome during lecture
- A 28 yo M with all the physical findings of Marfan Syndrome is found to test negative for the disorder at Mayo Clinic in 2015
- A 21 yo standout Division I basketball player is found to have Marfan's Syndrome and is disqualified from the NBA Draft.

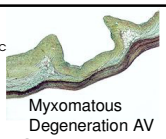
41

Marfan's Syndrome

- Autosomal Dominant
- 1 in 10,000
- Mutation in "fibrillin" gene, FBN1 (extracellular matrix glycoprotein)
- Connective Tissue Disorder
- Characteristic physical findings (Tall, thin, elongated limbs, myopia (elongated globe), hypermobile joints
- Patients at risk for aortic dissection or aortic dilation and rupture

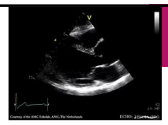
42

Marfan's Syndrome Characteristic Physical Findings



Myxomatous Degeneration AV

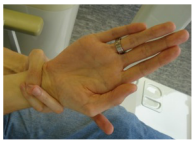
Lens Dislocation



Dilated aortic Root (diastolic murmur)

PLEIOTROPY

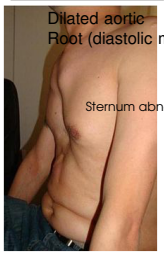
Arachnodactyly



Armspan >1.05 height

Elongated face

High Arched Palate



Sternum abnormalities

43

43

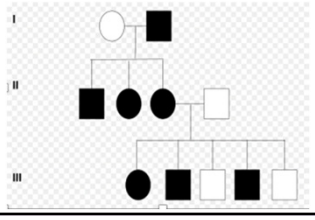
Isaiah Austin

- Considered to go in first round of NBA Draft 2014
- Diagnosis of Marfan's Syndrome precluded NBA Draft
- In middle school, he had retinal detachment
- Blind in R eye
- Kept that a secret
- Currently playing professional basketball in Philippines

44

Medical Student with physical findings

- What about family history (Autosomal Dominant)

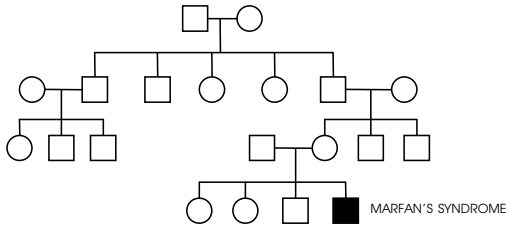


45

How could he have it if his parent's didn't?

We may need to employ Jerry Springer.

46



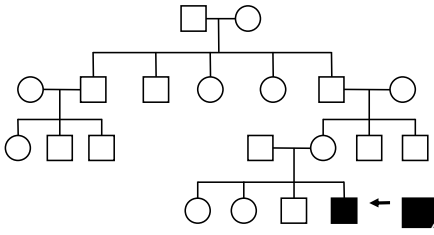
MARFAN'S SYNDROME

AUTOSOMAL RECESSIVE?

47

25-35% of patients with Marfan's Syndrome are the product of a NEW MUTATION

De novo mutation



New

48

28 yo M with Marfanoid habitus

- All cardiac imaging normal.
- FBN-1 mutation testing was negative.
- Physician Recommendations: "He does not have marfan syndrome and he does not require surveillance of the aorta."
  - Attributed habitus to "connective tissue disorder"

49

Criteria for diagnosis of Marfan's

In the presence of a family history of Marfan's Syndrome, and seven points from below criteria

Wrist AND Thumb sign (3 points)	Wrist OR Thumb sign (1 point)
Pectus carinatum (2 points)	Pectus Excavatum (1 point)
Hindfoot Deformity (2 points)	Pes Planus (1 point)
Pneumothorax (2 points)	Dural ectasia (1 point)
Protrusion acetabuli (2 points)	Scoliosis (1 point)
Reduced Elbow Extension (1 point)	Facial Features (1 point)
Skin striae (1 point)	Myopia (1 point)
Mitral Valve Prolapse (1 point)	Increased arm span/height (1 point)

50

Criteria for diagnosis of Marfan's

In the presence of a family history of Marfan's Syndrome, and seven points from below criteria

Wrist AND Thumb sign (3 points)	Wrist OR Thumb sign (1 point)
Pectus carinatum (2 points)	Pectus Excavatum (1 point)
Hindfoot Deformity (2 points)	Pes Planus (1 point)
Pneumothorax (2 points)	Dural ectasia (1 point)
Protrusion acetabuli (2 points)	**Scoliosis (1 point)**
Reduced Elbow Extension (1 point)	Facial Features (1 point)
Skin striae (1 point)	Myopia (1 point)
Mitral Valve Prolapse (1 point)	Increased arm span/height (1 point)

51

Marfan's Syndrome

- It's important for the clinician to be able to "screen" for marfan's syndrome with the **PHYSICAL EXAM** as this is a ***CLINICAL DIAGNOSIS***
- Family History and Past Medical History plays a role
- "Confirmation of marfan syndrome by identification of mutations in FBN1 is not currently practical because extreme **allelic heterogeneity** makes identification of the causative mutation in each family prohibitively labor-intensive and because of the lack of reliable genotype-phenoptye correlation. Mutational analysis is neither fully sensitive nor specific for Marfan Syndrome, limiting clinical utility."

52

HUNTINGTON DISEASE

- HUNTINGTON DISEASE is a progessive neurodegenerative disorder caused by mutations in the HD gene for protein "huntingtin" which is inherited in an Autosomal Dominant fashion.
- The course is one of slow but relentless deterioration in cognitive and motor function.
- There is currently **no effective treatment** for Huntington Disease

53

Huntington Disease

- Prevalence: 0.1 to 7 per 100,000.
- Caused by **TRINUCLEOTIDE REPEAT EXPANSION** on Chromosome 4 of the Huntingtin gene
- Normal allele DNA: (------**CAGCAGCAGCAGCAGCAG**-------)
  - (CAG repeats number less than 36)
- ABNORMAL allele DNA: (------ **CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG CAGCAGCAGCAGCAG CAGCAGCAGCAGCAGCAGCAG CAGCAGCAGCAGCAGCAGCAG**------)
  - (CAG repeats number more than 36)

54

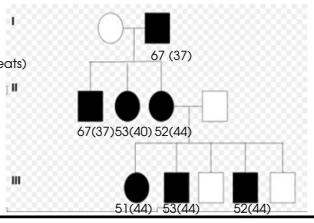
Huntington Gene

- Identified early through linkage analysis
- The disease is **100% penetrant**, meaning everyone who gets the disease gene gets the disease phenotype (clinical symptoms)
- Delayed age of onset - Difficult to identify affected individuals at young age
- Anticipation - Disease severity changes from generation to generation
  - It is possible for the trinucleotide expansion to get longer if passed from father to child.

55

Huntington Inheritance

Age diagnosis (CAG repeats)



Anticipation:
Disease gets more severe (earlier onset) from Generation to generation

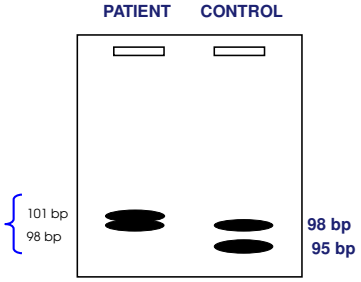
56

Genetic Test for Huntington's

- Take DNA sample from patient
- Isolate TWO copies of chromosome 4
- Amplify the genes through PCR (Polymerase Chain Reaction)
- Electrophorese the two genes in Agarose Gel

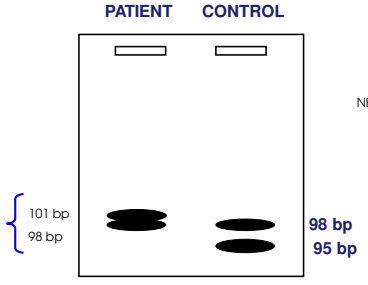
57

SOUTHERN BLOT TEST for HUNTINGTON



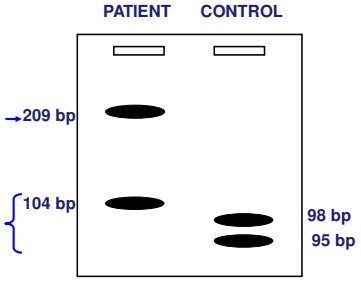
58

SOUTHERN BLOT TEST for HUNTINGTON



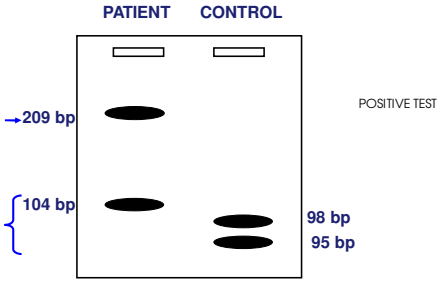
59

SOUTHERN BLOT TEST for HUNTINGTON



60

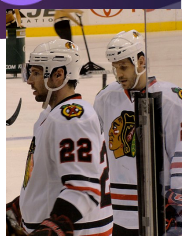
SOUTHERN BLOT TEST for HUNTINGTON



61

Jake Dowell

- Professional Hockey Player
- Father and Brother affected with Huntington's disease
- Struggled for years whether or not to have genetic testing
- Eventually tested negative



62

Huntington's Disease

- 46 yo F with Huntington's Disease of moderate severity with an 8 year old daughter. Patient received the disease from mother.

63

Punnett Square

FATHER (unaffected)

MOTHER (affected)	h	h
H	Hh	Hh
h	hh	hh

64

Punnett Square

FATHER (unaffected)

MOTHER (affected)	h	h
H	Hh	Hh
h	hh	hh

Recurrence Risk Would be 50%

Recurrence Risk is Likelihood child Will HAVE THE DISEASE PHENOTYPE

65

What if your patient brings her daughter in to be tested?

- Children's autonomy, their ability to make decisions for themselves about their own genetic constitution, must also now be balanced with the desire of parents to obtain genetic information.
- The patient's AUTONOMY needs to be considered
- Beneficence and **non-maleficence** needs to be considered

66

Alpha-1 antitrypsin deficiency

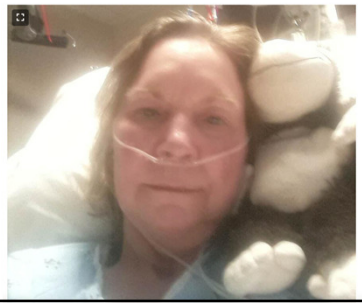
- 57 yo F new to your practice was just hospitalized with respiratory failure. She left the hospital with a diagnosis of homozygosity "ZZ" for alpha-1 antitrypsin deficiency and she has a severe obstructive lung defect.
- Her daughter comes with her and asks to be tested. She is 36 years old and she smokes ½ pack of cigarettes per day.

67

Alpha-1 antitrypsin

- Alpha-1 antitrypsin is a protein normally produced modulates the inflammatory response
- Alpha-1 antitrypsin is a protease inhibitor that prot from enzymes such as elastase
- Deficiency in active protein dramatically increase emphysema, especially in smokers

68



69

Alpha-1 Antitrypsin results

GENOTYPE	AAT (PROTEIN) levels
MM (Normal)	88-275 mg/dL
MS (Not Deficient)	86-270 mg/dL
SS (Mildly Deficient)	60-223 mg/dL
MZ (Mildly Deficient)	50-222 mg/dL
SZ (Moderately to Severely Deficient)	32-144 mg/dL
ZZ (Severely Deficient)	19-39 mg/dL

**Genotypic analysis by allelic discrimination detects the S and Z alleles which Constitutes >99% (>90%?) of all mutations that cause alpha-1 antitrypsin deficiency.

70

57 yo F prior smoker

- AAT Genotype **ZZ**
- Level AAT **37.4 mg/dL**
- **PEF1** **20% predicted (Severe obstructive lung disease)**
- **Currently considering lung transplant**

71

36 yo daughter

- AAT Genotype **ZZ**
- Level AAT **23.3 mg/dL**
- **PEF1** **90% predicted**
- **Currently smoking**

72

Recommendation for AAT testing

- It is estimated 80,000-100,000 people severe deficiency of AAT
- All adults with persistent airflow obstruction on post-bronchodilator spirometry should be tested for AATD
- Severe disease can be treated with infusions of pooled human alpha-1 antitrypsin (AAT)

73

Direct to Consumer Genetic Testing

- 23 and me
- CRI Genetics
- Family Tree DNA
- Living DNA
- Ancestry.com
- Futura Genetics
- Navigenics

74

24 yo medical student

- Had Direct to Consumer Genetic Testing "23 and me"
- Tested positive as a Carrier of ApoE4 allele for Alzheimer's Disease
- Alzheimer's demonstrates **MULTIFACTORIAL INHERITANCE** pattern; genetics and environment play a role

75

23 and me

- "Direct to consumer genetic testing" was named "Invention of the Year" by TIME MAGAZINE in 2008
- Valuation of the company is about $1.8 billion
- Holiday sale! Health and ancestry **$139**, ancestry only **$69**
- Tests for genetic mutations that put the patient at risk for certain diseases, including Alzheimer's, Parkinson's, Alpha-1 antitrypsin deficiency, glucose-6 phosphate dehydrogenase deficiency, Gaucher's disease
- American Alzheimer's association **does not currently recommend genetic testing** because there are no treatments and if someone has the genetic tendency, it doesn't mean they are going to get the disease

76

23 and me

- March, 2018, FDA approved 23 and me to perform BRCA testing
- 3 variants of BRCA1 and BRCA2 are tested, currently
- "Our test does not account for all genetic variants that can cause a higher risk of cancer, and people should continue with their recommended cancer screenings."

77

Direct to Consumer Genetic Testing

- Accuracy?
- Interpretation?
- Value?

78

Applications of genetic diagnosis

- Prevention
- Carrier detection in recessive diseases
- Diagnosis for late-onset diseases
- Diagnosis for diseases with incomplete penetrance
- Prenatal diagnosis:
  - Amniocentesis
  - Chorionic villus sampling
- Prenatal diagnosis in cases where there is a history of a genetic disease in one/both parent's family(ies).
- Treatment

79

Privacy of Genetic Testing

- Genetic Information Non-Discrimination Act
- "federal law that protects individuals from genetic discrimination in health insurance and employment."
  - Health Insurers
  - Life Insurance, Disability insurance or long-term care insurance

80

Role of the Primary Care Physician

- Family History
- Physical Exam
- Testing and counseling for common diseases
- Referral
- Medical Home

81

Cost of Genetic Testing

- Karyotype $500
- Exome sequencing $9,000

82

Future of genetic testing

- Enhanced application of genetic testing to apply to common diseases demonstrating multifactorial inheritance (Diabetes, Hypertension, heart disease)
- Pharmacogenomics
  - The study of how an individual's genetics affects their response to drugs.
  - Example: HER2 receptor in breast cancer

83

Websites

- OMIM – www.ncbi.nlm.nih.gov/omim
- www.geneclinics.org
- www.medicalhomeinfo.org/gpci

84

References

- *Genetics in Medicine.* Nussbaum, McInnes and Willard, Edition 7. 2007.
- *The Gene* Siddhartha Mukherjee
- National Institutes of Health, U.S. National Library of Medicine Genetics Home Reference
- http://www.onlinejacc.org
- UpToDate
- USPSTF website

85

THE END

86