Advances in the Genetic Diagnosis of the Cerebellar Ataxias

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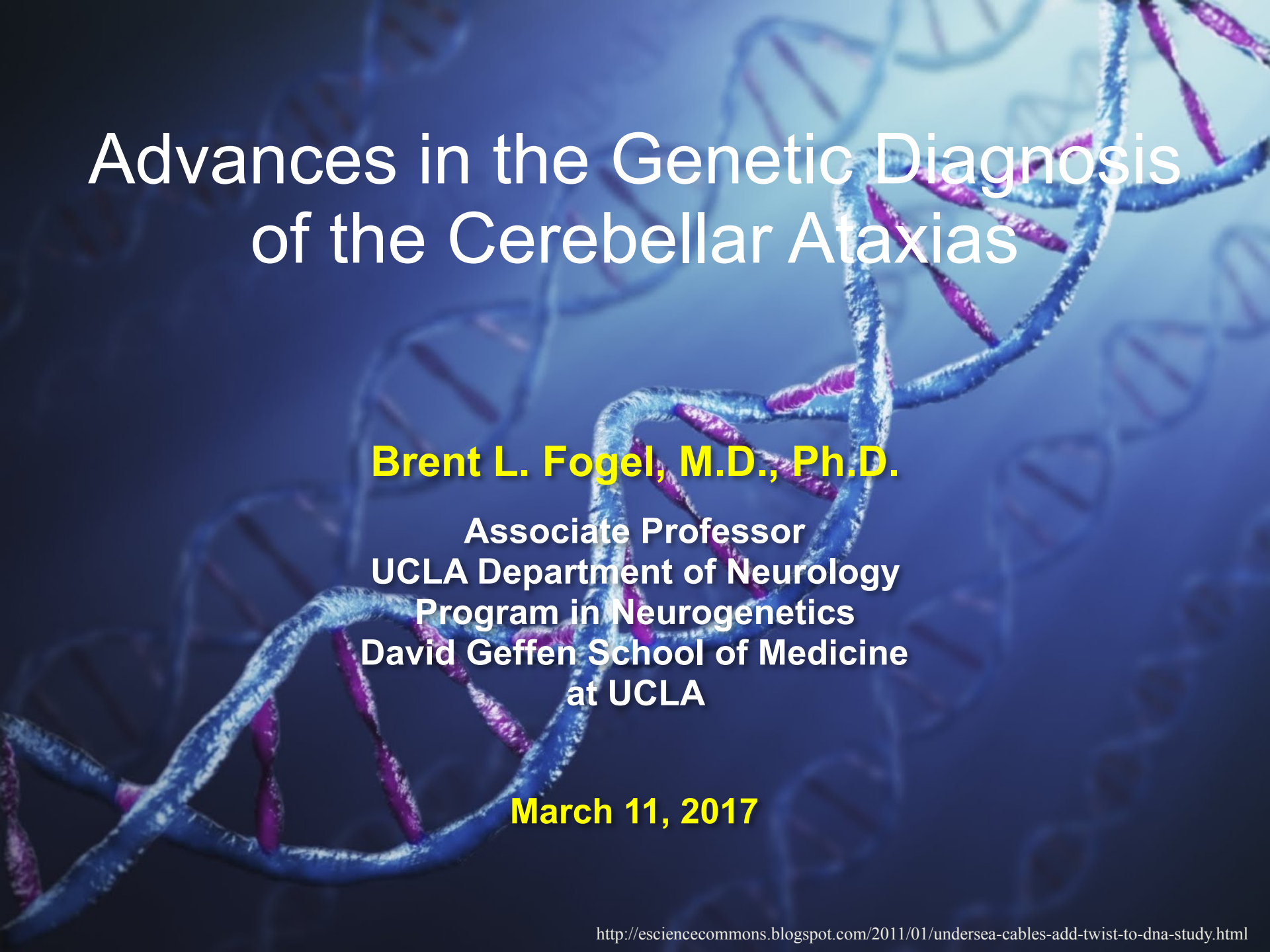

http://esciencecommons.blogspot.com/2011/01/undersea-cables-add-twist-to-dna-study.html

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DISCLOSURES

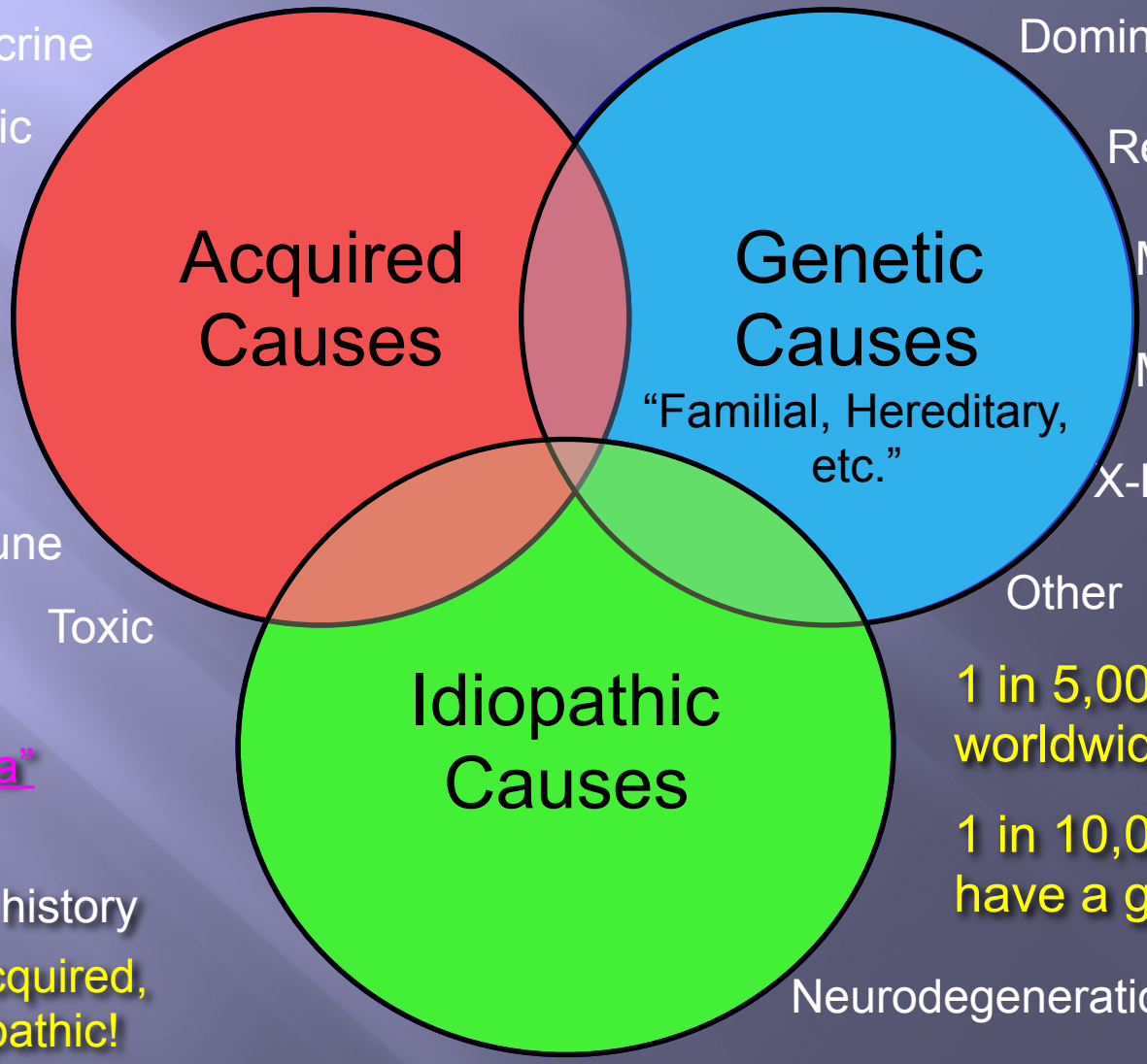
- Dr. Fogel receives funding from the National Institutes of Health.
- Dr. Fogel is employed at an academic medical institution that offers diagnostic clinical exome sequencing. Dr. Fogel has no financial relationships related to this testing.
- Dr. Fogel has no personal financial relationships with commercial interests relevant to this presentation during the past 12 months to disclose or list.

Ataxia is a symptom…NOT a disease!

Term provides no information on cause, severity, or prognosis

- Upper respiratory Infection (viral) (“a cold”)
- Influenza (viral) (“the flu”)
- Pneumonia (bacterial or viral)
- Tuberculosis (mycobacterial)
- Coccidioidomycosis (fungal) (“Valley Fever”)
- Ebola (viral)

The Importance of a Thorough Medical Evaluation: Many Causes of Cerebellar Ataxia!



"Sporadic Ataxia"

Unexpected

No clear family history

…but can be acquired, genetic, or idiopathic!

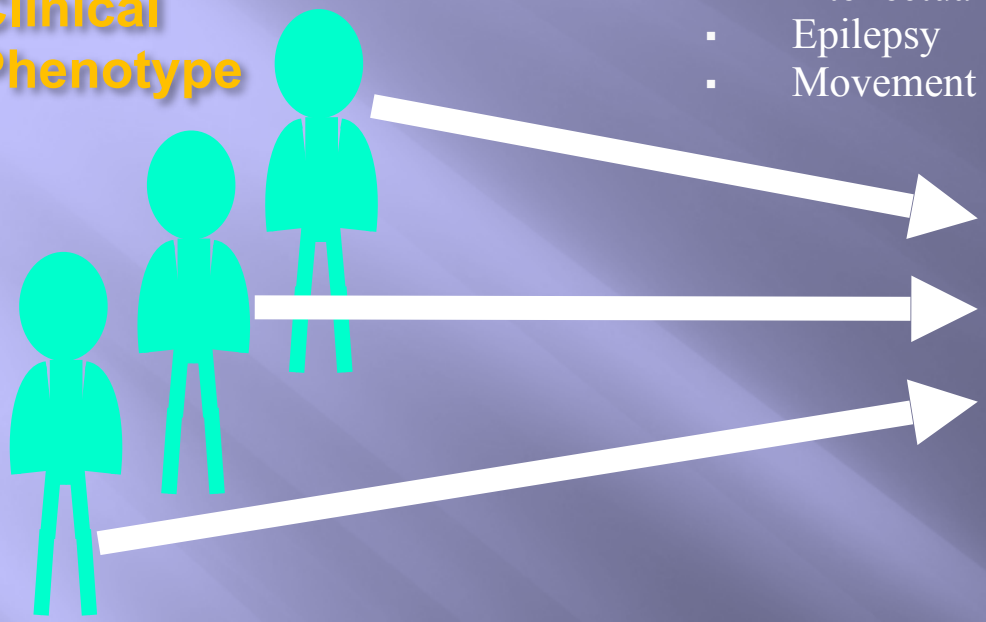
1 in 5,000 persons worldwide have ataxia

1 in 10,000 persons have a genetic ataxia

Diagnostic Reservoirs Hiding Neurogenetic Disease

- Cerebral palsy
- Intellectual disability
- Epilepsy
- Movement disorders
- **Ataxia**
- Dementia
- Multiple sclerosis
- Peripheral neuropathy

Shared Clinical Phenotype

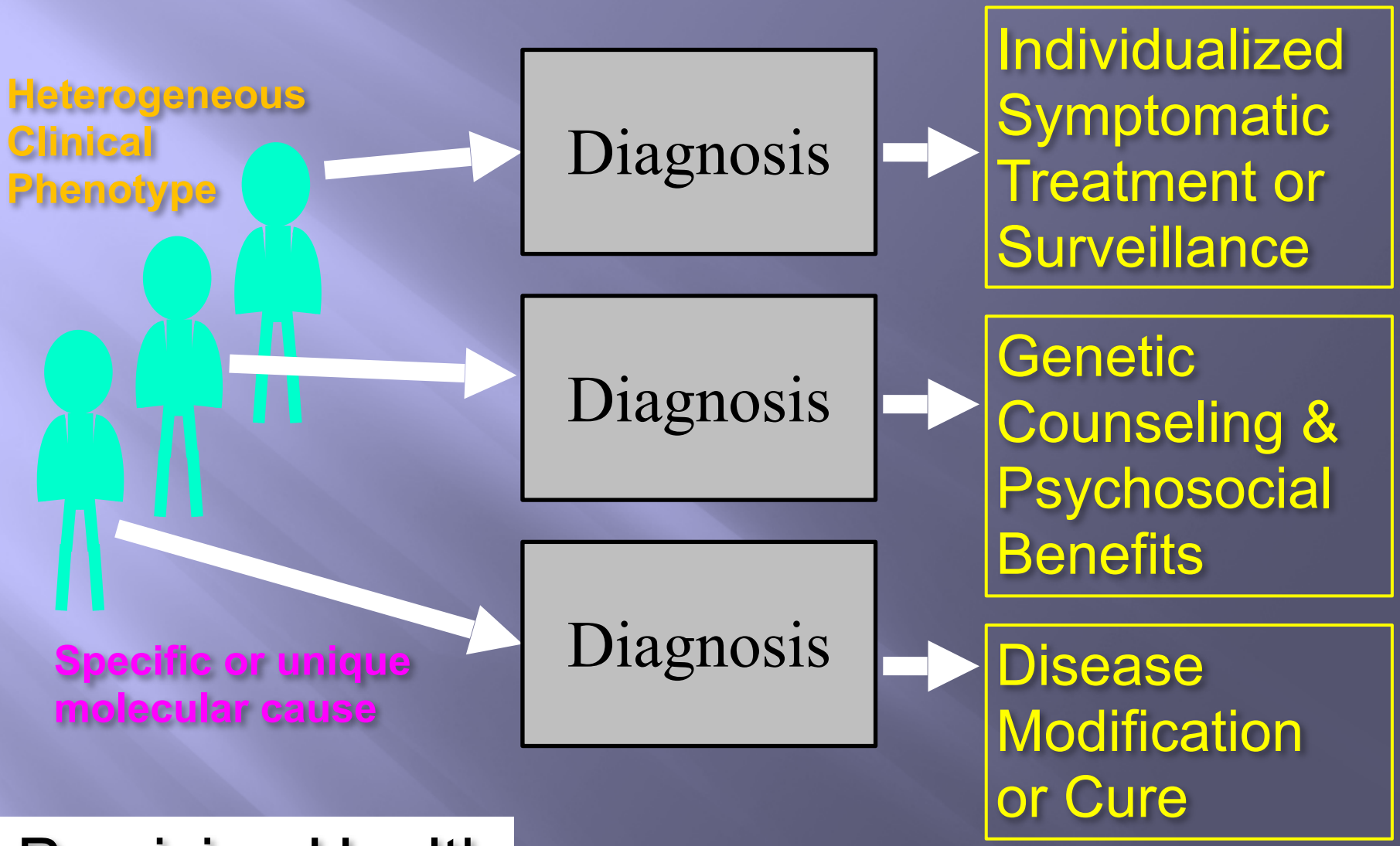


Diagnosis

Presumed identical or similar cause

Same Management for Everyone?

Traditional Medical Approach



Precision Health Approach

Ataxia is Common in Neurogenetic Disease

More than **680** genes are associated with ataxia as a primary or secondary symptom*

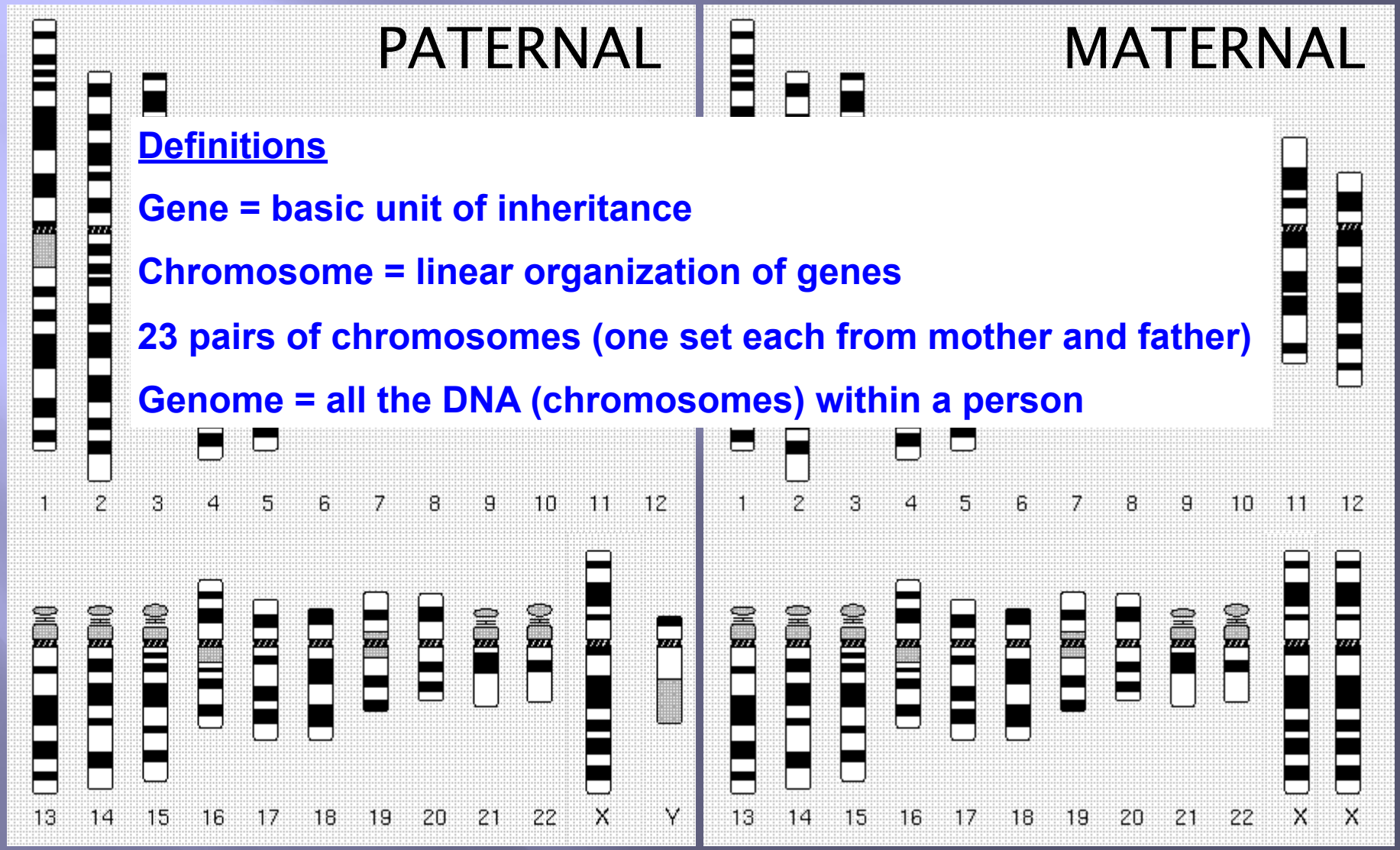
Total Genes	Inheritance
~ 396	Autosomal Recessive
~ 229	Autosomal Dominant
~ 46	X-linked
~ 12	Mitochondrial

(…and estimates suggest that we currently only know about half of the genes that cause hereditary ataxia!)

Effective strategies are necessary for optimal clinical evaluation.

*Source: Online Mendelian Inheritance in Man, OMIM®. 11/2016. http://omim.org/

DNA & Genetics - Chromosomes

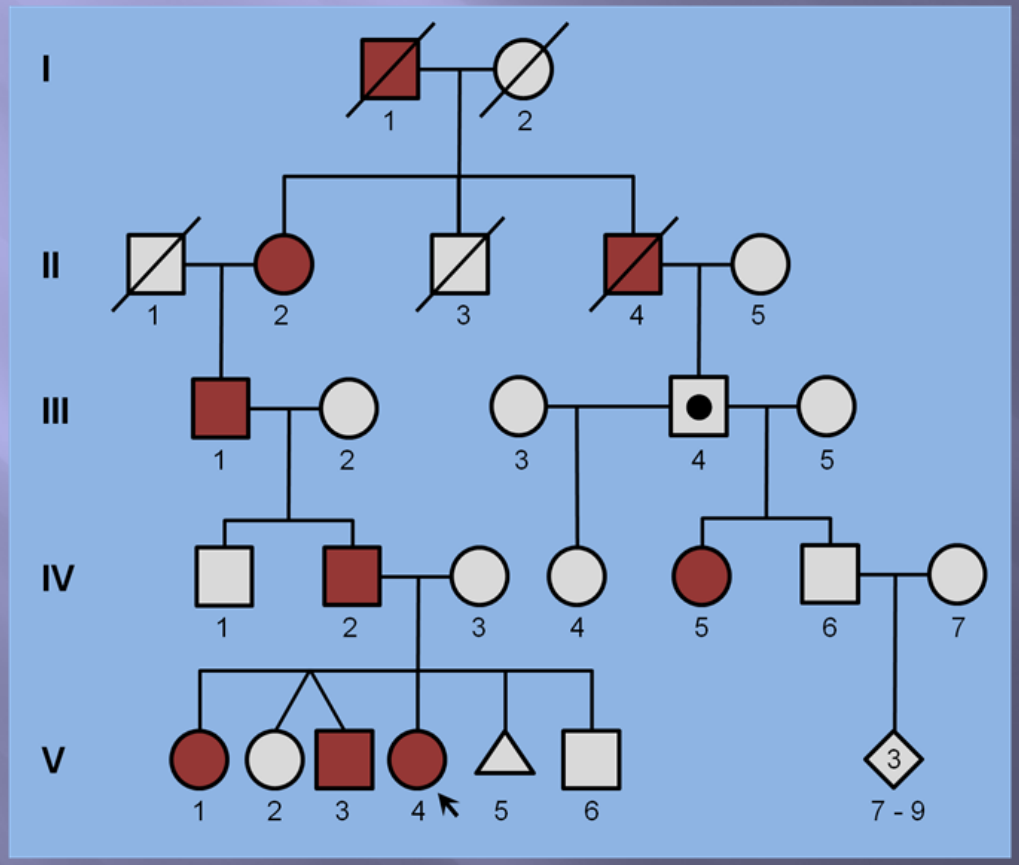


David Adler, University of Washington

DNA & Genetics - Inheritance

The Autosomal Dominant Cerebellar Ataxias

- Commonly referred to as the Spinocerebellar Ataxias (SCAs)
- Phenotype of slowly progressive, clinically heterogeneous ataxia
- Currently 43 distinct clinical forms with 30 identified genes



Fogel and Geschwind, Neurology in Clinical Practice, 2012

Autosomal Dominant Cerebellar Ataxia

- Adult onset, typically between 20-50 years of age
- Overall ~ 4 cases per 100,000 people worldwide

Most Common

SCA3

SCA1

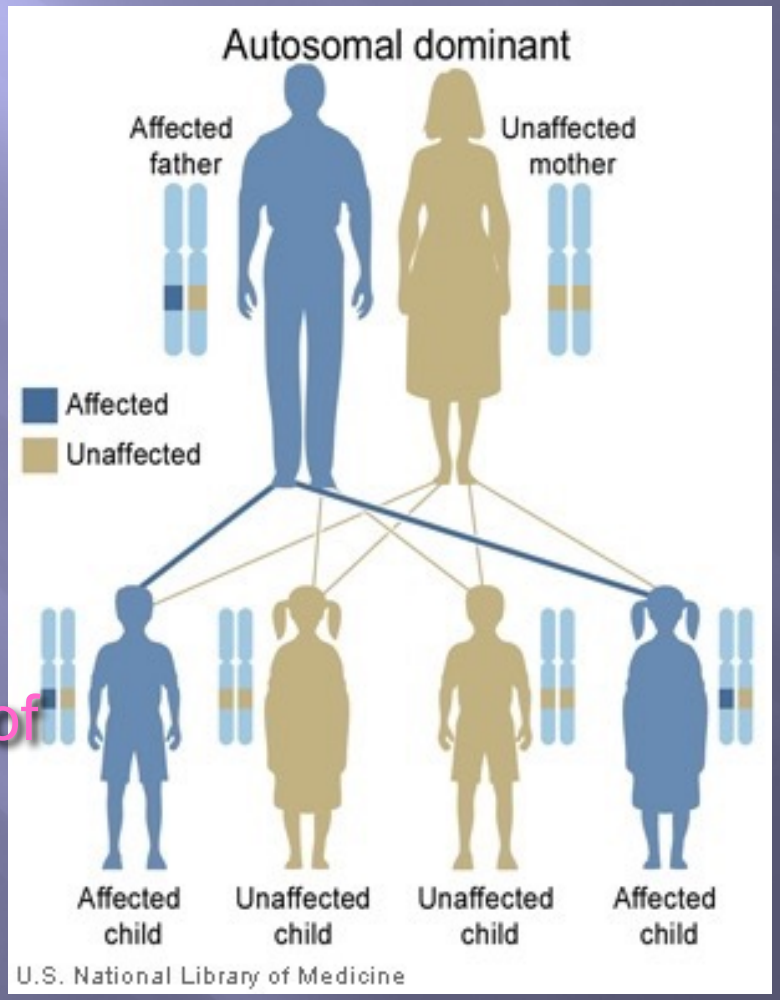
SCA2

SCA6

SCA7

(~50% of total)

Inherit **one** "damaged" copy of the gene (from either mom or dad)



http://ghr.nlm.nih.gov/handbook/illustrations/autodominant

Most Recent

2014

SCA21 (France)

SCA34 (Canada)

SCA38 (Italy)

SCA40 (China)

2015

SCA41 (USA)

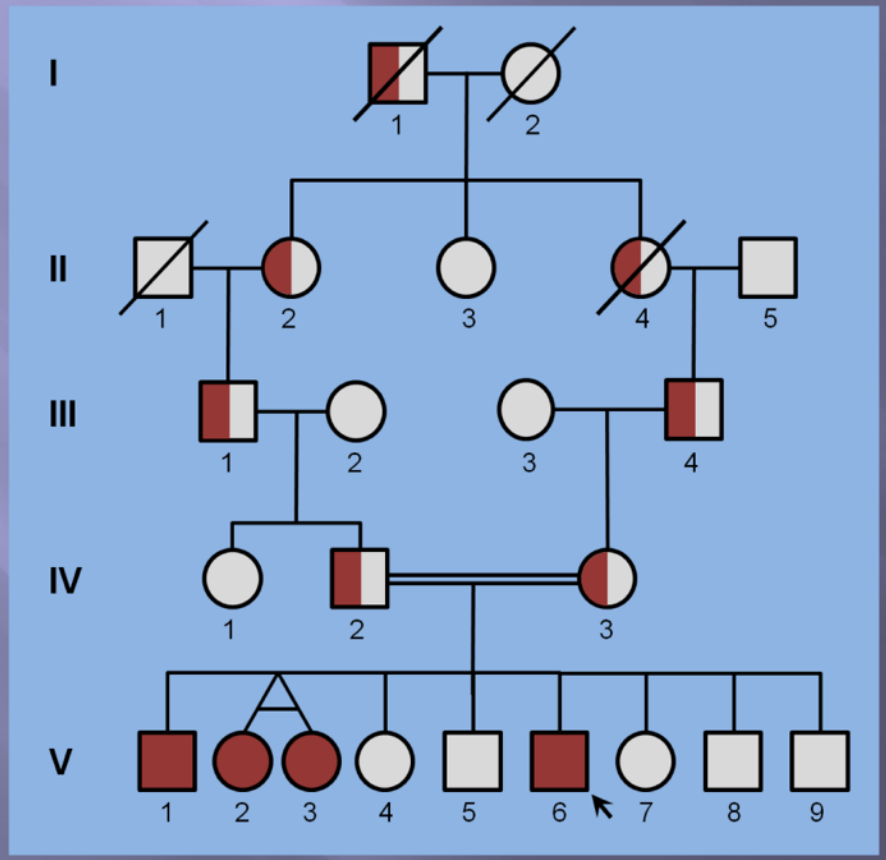
SCA42 (France, Japan)

2016

SCA43 (Belgium)

The Autosomal Recessive Cerebellar Ataxias

- New diseases being named Spinocerebellar Ataxia, Recessive (SCARs)
- Slowly progressive often with sensory/sensorimotor polyneuropathy
- Several diseases involve organ systems outside the CNS (biomarkers)
- At least 40 identified genes cause primary recessive ataxia



Fogel and Geschwind, Neurology in Clinical Practice, 2012

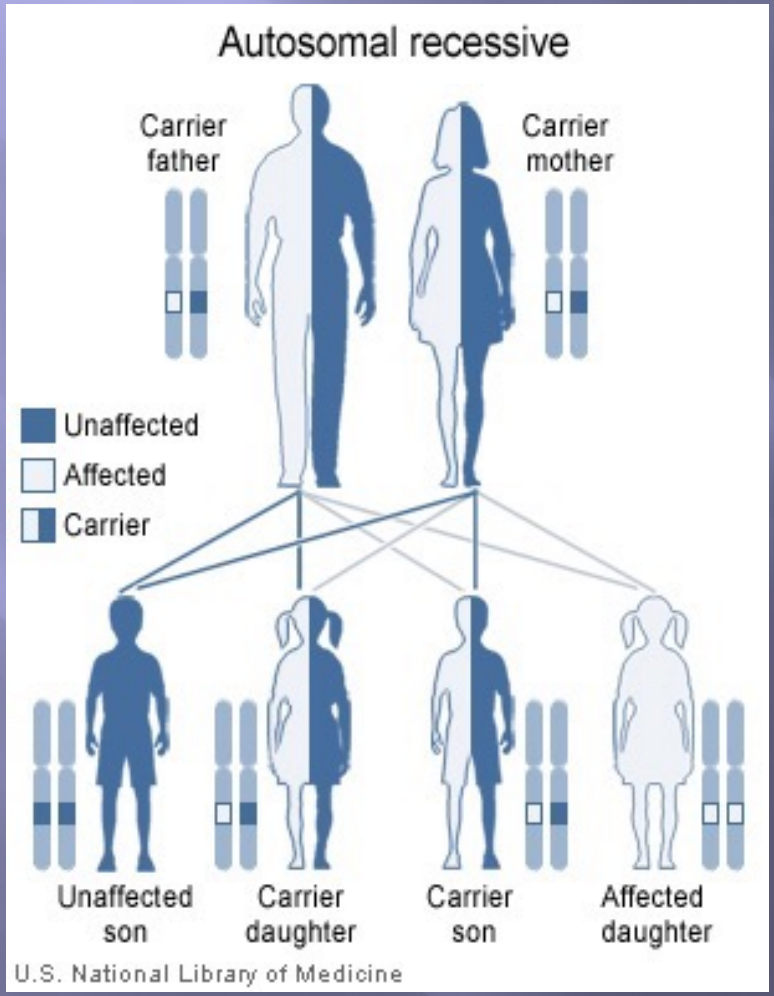
Autosomal Recessive Cerebellar Ataxia

- Onset typically before age 20 years
- Milder variants can present in adulthood
- Overall ~ 4 cases per 100,000 people worldwide

Most Common

Friedreich ataxia (~50%)

Inherit **two** "damaged" gene copies (one from mom & dad)



http://ghr.nlm.nih.gov/handbook/illustrations/autorecessive

Most Recent

2014

SCAR17 (Turkey, Netherlands)

SCAR20 (Portugal, Middle East)

SCAR23 (Ireland, Egypt)

2015

SCAR19 (Turkey)

SCAR21 (Europe, Cuba)

2016

SCAR22 (Japan)

SCAR24 (China)

Genetic Testing – Types of Genetic Testing

Full gene sequencing (Traditional Sanger method)

- Most complete test but also most expensive per gene
- Can potentially discover novel coding mutations
- REMEMBER: Not every sequence change causes disease!

Targeted mutation analysis

- Less expensive, useful in families to detect pre-defined mutations
- REMEMBER: Negative test rules out the specific mutations only!

Repeat expansion testing

- Required for common dominant SCAs and Friedreich Ataxia
- Cannot identify sequence changes or other types of mutations

Genetic Testing – Traditional Gene Panels

Often combine multiple types of testing for several different genes

- Full gene sequencing (Traditional Sanger method)
- Targeted mutation analysis
- Repeat expansion testing

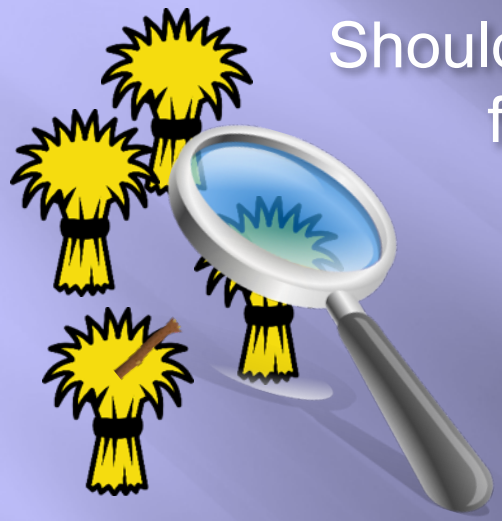
Can be very expensive per gene

- Range US$500 - US$30,000 or more
- Insurance coverage varies

These panels don't test every ataxia gene

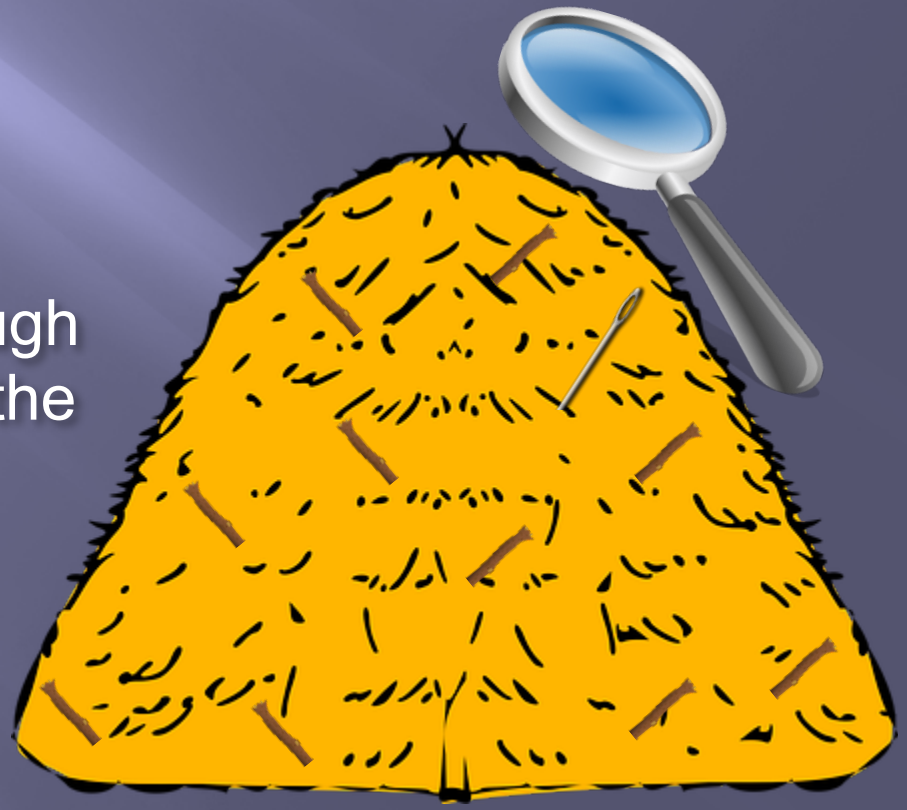
- Not all ataxia genes are known!
- Not all genes have specific tests
- Some genes only cause ataxia rarely (e.g., not in all patients) so they aren't included

Genetic Testing Bias



Should you look at hay by the handful for anything that might be sharp?

…or should you look through the whole haystack for the needle?



Classic Question: Single or Multi-Gene Panel?

Major Caveat to Biased Single Gene or Multi-Gene Panel Testing

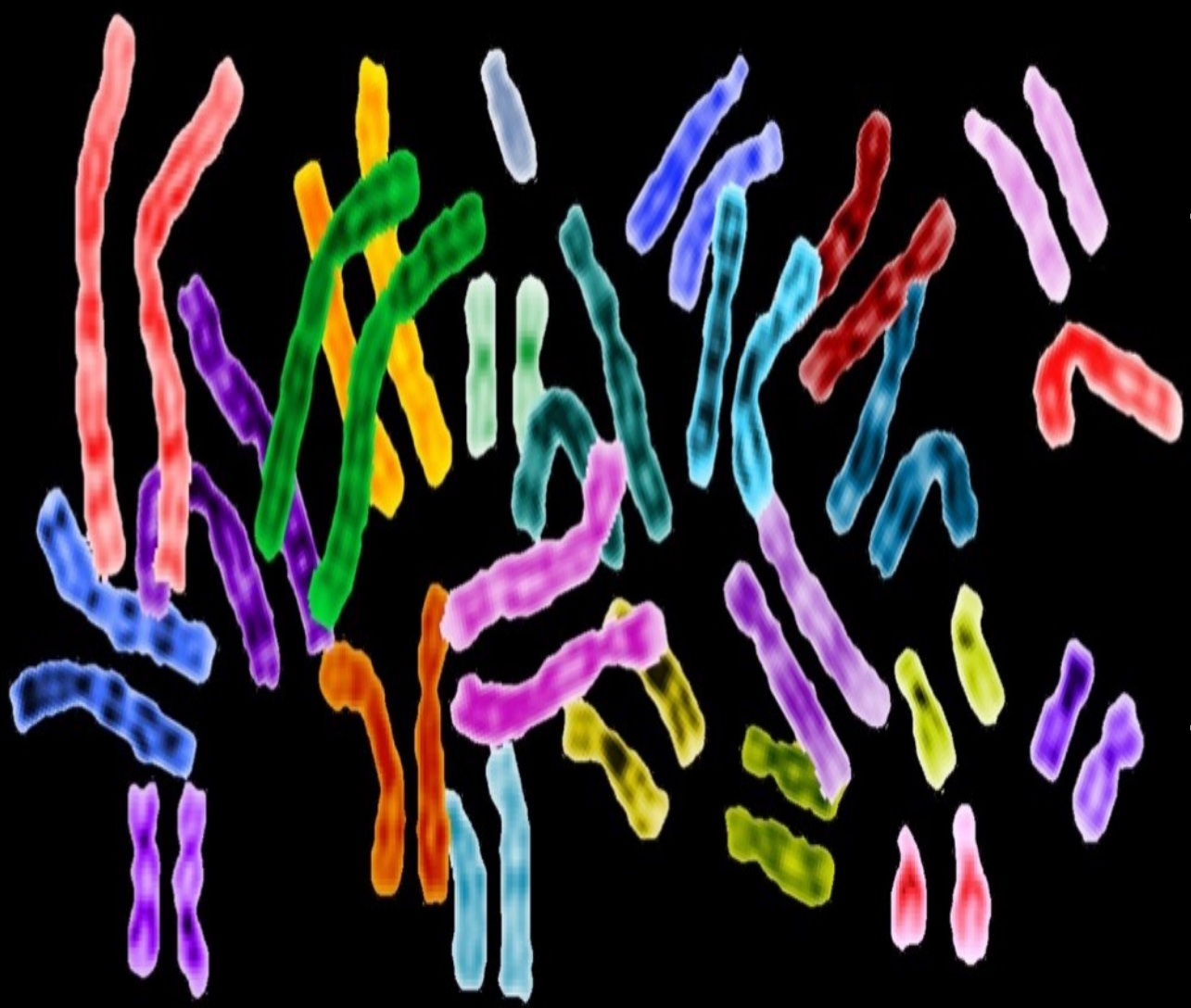
- Clinical Heterogeneity: The same phenotype common to one disorder may be an atypical form of another, how do you know?
- Genetic Expressivity: Currently documented phenotypes may not represent the only forms of disease caused by a gene.

Examples:

- Late-onset Friedreich Ataxia (up to 25% of cases)
- Fragile X-Syndrome & Fragile X-Tremor/Ataxia Syndrome (premutation)
- Adult Polyglucosan Body Disease & Glycogen Storage Disease Type IV
- X-linked Adrenoleukodystrophy & Adrenomyeloneuropathy
- AOA2 (ataxia & polyneuropathy) vs. ALS4 (motor neuron)

How can one minimize such confounders and maximize genetic testing efficacy?

Exome Sequencing: An Unbiased Tool for Diagnosis



Genome
3.3 x 10^9 base pairs
~20,000 genes

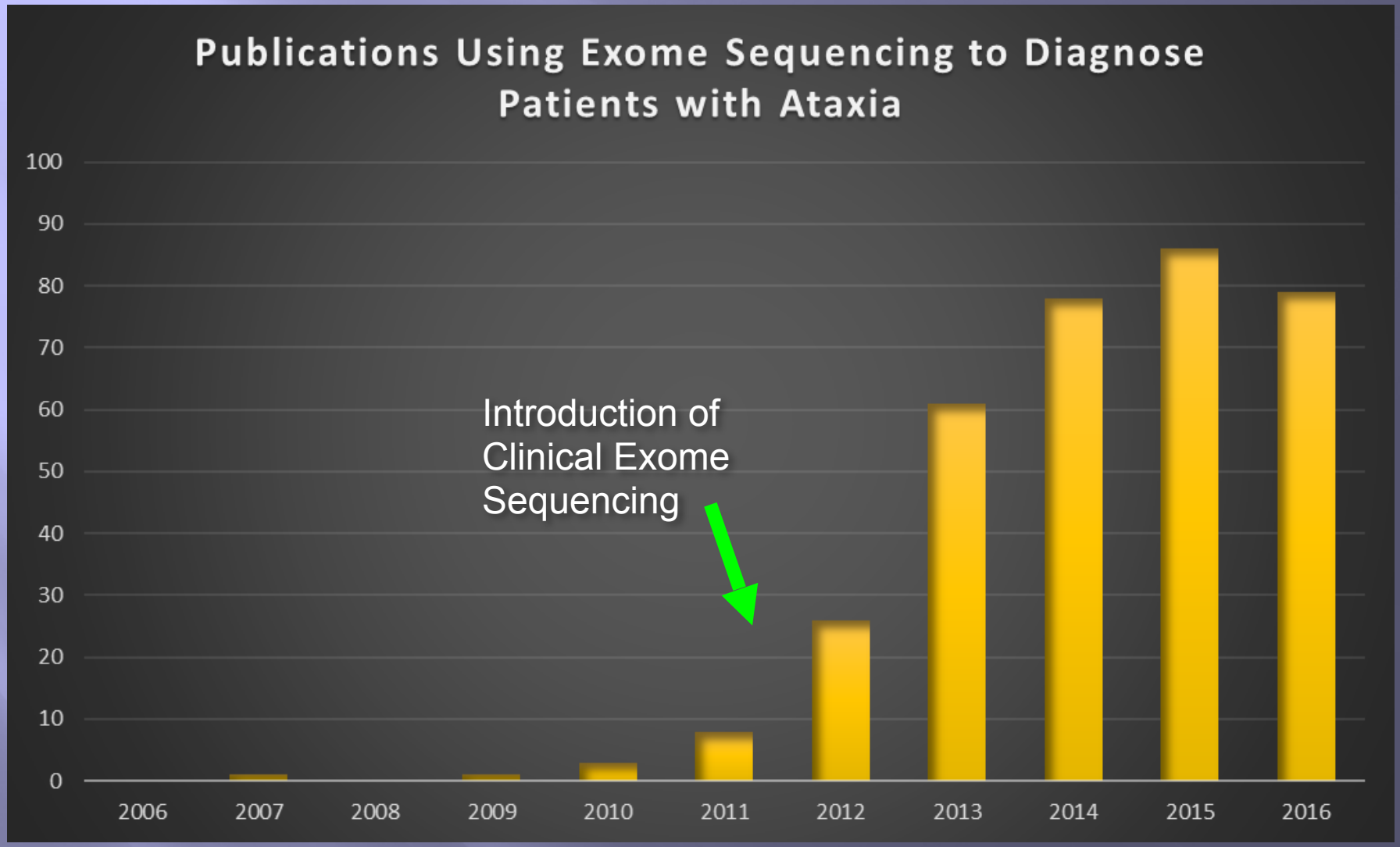
Exome
~1% of genome
~3 x 10^7 base pairs
~20,000 genes

Examination of every gene simultaneously provides an unbiased method of genetic testing

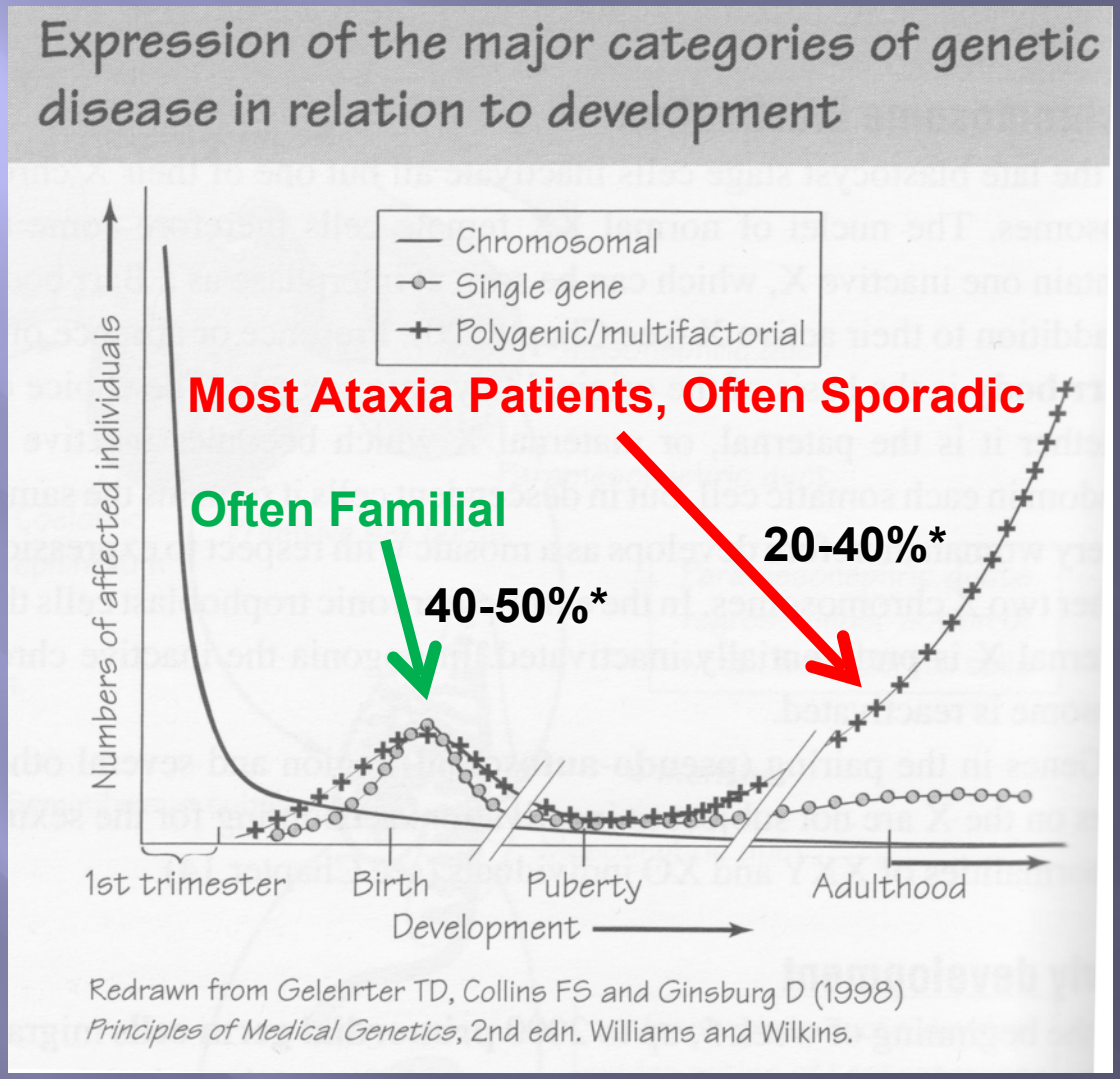
~26% overall diagnostic rate
(~3,000 neurologic cases)

http://www.genome.gov/dmd/index.cfm?node=Photos/Graphics

The Rise of Exome Sequencing in the Diagnosis of Ataxia



When to Use Exome Sequencing in Ataxia?



*Patients with negative work-up for acquired causes and common genetic causes

Pritchard DJ & Korf BR 2003 Medical Genetics at a Glance

Types of Next-Generation Genetic Testing

Whole Exome (sometimes called a "Clinical Exome")

- Most complete test, covers all ~20,000 genes in the genome
- Most expensive overall (~$5-10K) but least expensive per gene

Next-Generation Panels
(sometimes called "Exome Panels" or even a "Clinical Exome")

- Less expensive per gene than traditional Sanger panels
- Includes only a few to hundreds of genes depending on the test
- Some laboratories may offer reflex option to whole exome if negative

REMEMBER!

- Different laboratories may analyze and/or report results differently
- Method does not detect repeat expansion disorders
- Not every sequence change causes disease

Which Type of Next-Gen Sequencing Test is Best for Ataxia?

PATIENT POPULATION STUDIED	TOTAL PATIENTS OR FAMILIES	POSITIVE GENETIC RESULT	DIAGNOSTIC RATE	REFERENCE
Next-Generation Panel Testing (Few Genes)				
Ataxia (age <50 years or familial onset)	50	9	**18%**	Németh et al. 2013
Exome Capture Filtered for Gene Panel Testing (Many Genes)				
Ataxia (age <45 years or familial onset)	28	9	**32%**	Van de Warrenburg et al. 2016
General Whole Exome Sequencing ("Ataxia" Listed as a Symptom)				
Ataxia (symptom only)	86	11	**13%**	Lee et al. 2014
	59	26	**44%**	Farwell et al. 2015
Ataxia-Specific Whole Exome Sequencing Studies (Common Gene Testing Negative)				
Ataxia (all patients) (mostly sporadic & adult onset)	76	16	**21%**	Fogel et al. 2014
Ataxia (adult onset)	12	4	**33%**	Keogh et al. 2015
Ataxia with cerebellar atrophy (childhood onset)	23	9	**39%**	Ohba et al. 2013
Ataxia (all patients) (mostly childhood & familial onset)	22	9	**41%**	Pyle et al. 2015
Ataxia (childhood onset)	28	13	**46%**	Sawyer et al. 2014
"Mini"-Exome (~25%) Filtered for Gene Panel Followed by All Genes & Copy Number Variation Analysis				
Ataxia (all patients) (mostly sporadic & childhood onset)	33	14	**42%**	Marelli et al. 2016

Redefining Phenotypes to Improve Diagnosis

Hereditary Spastic Paraplegia (HSP)

- Class of disorders characterized by progressive weakness and spasticity of the legs
- Prevalence roughly equal to Spinocerebellar Ataxia worldwide
- Genes designated as Spastic Paraplegia (SPG), now up to SPG77

SPG7

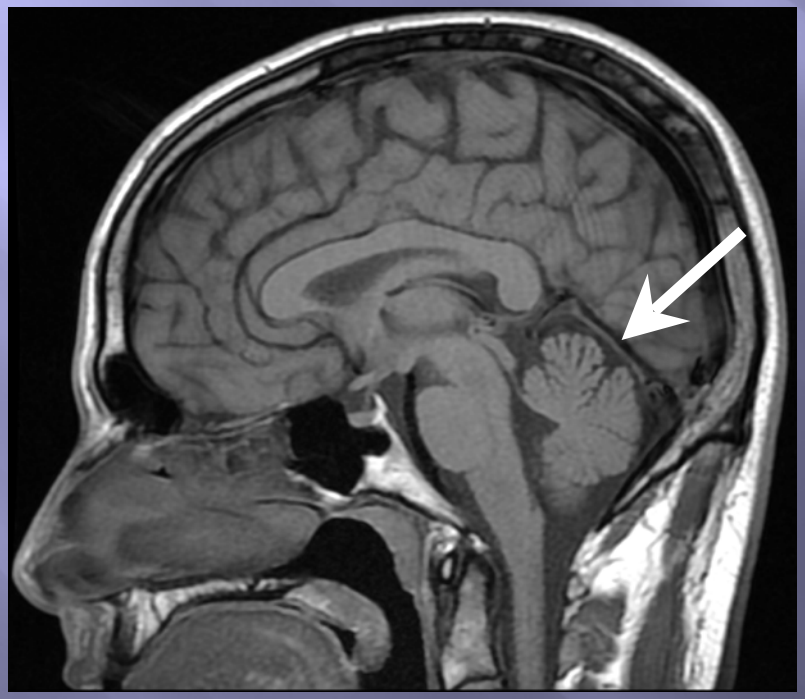
- Causes up to 12% of all recessive HSPs worldwide
- SPG7 has been identified in ataxia patients in several whole exome studies
- 39% (12/31) families in study of spastic ataxia in Canada (Choquet et al. 2015)

PNPLA6/SPG39

- Identified in 2008 in patients with spastic paraplegia
- In 2014 the SPG39 gene was found to cause forms of cerebellar ataxia including syndromes with visual and/or hormonal problems (Synofzik et al. 2014)
  - Boucher-Neuhäuser syndrome
  - Gordon Holmes syndrome
  - Laurence-Moon syndrome

Discovering New Genetic Disorders

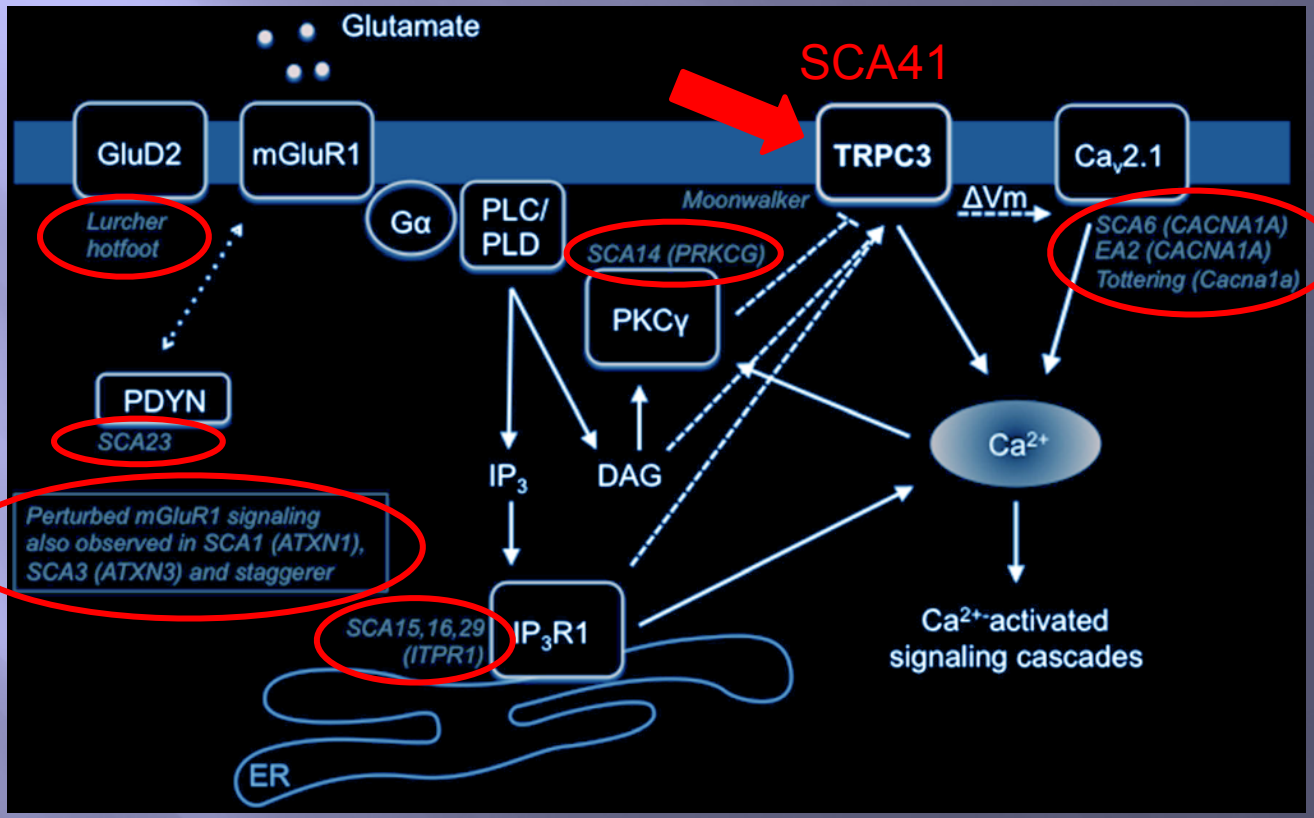
- 40 year old white man of European ancestry
- 2 years progressive imbalance and ataxic gait
- Negative evaluation for acquired causes of ataxia
- MRI with mild cerebellar vermian atrophy
- No family history but estranged from paternal side



Sagittal T1 magnetic resonance imaging of the brain shows very mild atrophy of the cerebellar vermis (arrow) with no brainstem involvement.

TRPC3

- TRPC3 is a non-selective cation channel linked to key signaling pathways affected in cerebellar ataxia



Role of TRPC3 in the mGluR1 signaling pathway essential for Purkinje cell function. Loss of any component in the depicted signaling cascade results in cerebellar ataxia in humans and/or mice.

Figure reproduced from Becker EB. Cerebellum 2015.

Rapid Identification of Treatable Patients

Clinical History

- 9 year old Lebanese girl with progressive balance problem since age 2 years
- Gait & limb ataxia, sensory neuropathy, areflexia and upgoing toes
- Scoliosis but no skin, cardiac, or muscle involvement
- Detailed genetic testing negative
- Exome sequencing identified homozygous mutations in *SLC52A2*
- *SLC52A2* encodes a membrane-bound riboflavin transport protein
- Mutation of *SLC52A2* causes Brown-Vialetto-Van Laere syndrome (juvenile-onset motor neuron disease, deafness, ataxia)
- Disease is typically fatal in 1st decade of life
- Identical mutation reported in classic BVVLS in 2 families (one from Lebanon)

Rapid Identification of Treatable Patients

Follow-Up

- **Patient diagnosed with Brown-Vialetto-Van Laere syndrome**
- Riboflavin transporter is defective but not absent, therefore could potentially drive uptake with high dose intake of riboflavin

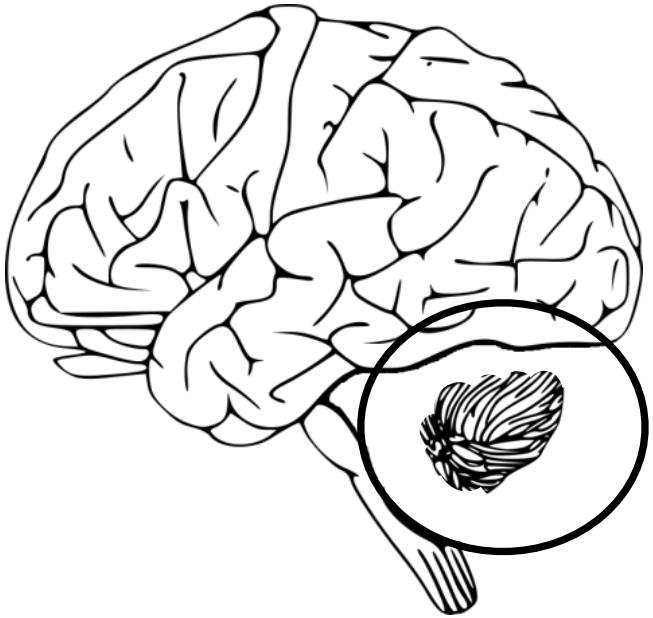
Treatment started immediately and symptoms stabilized.

Exercise and physical therapy led to marked improvements.

Now stabile for over 4 years.

Mildly clumsy but playing volleyball, dancing, karate, running long distance.

SHE IS ESSENTIALLY CURED.



Clinical Evaluation

Detailed History of Symptoms
Comprehensive Neurological Examination
Complete Family History
MRI of the Brain

Most common etiologies

Diagnostic Evaluation

Screen for Acquired Causes of Ataxia

Genetic etiologies

Common Genes:
Estimated 40-50%
of Genetic Ataxia

Basic Diagnostic: Initial Genetic Screening

Single Gene Testing*

Repeat Expansion Disorders

Dominant: SCA1, SCA2, SCA3, SCA6, SCA7

Recessive: FRDA

If negative

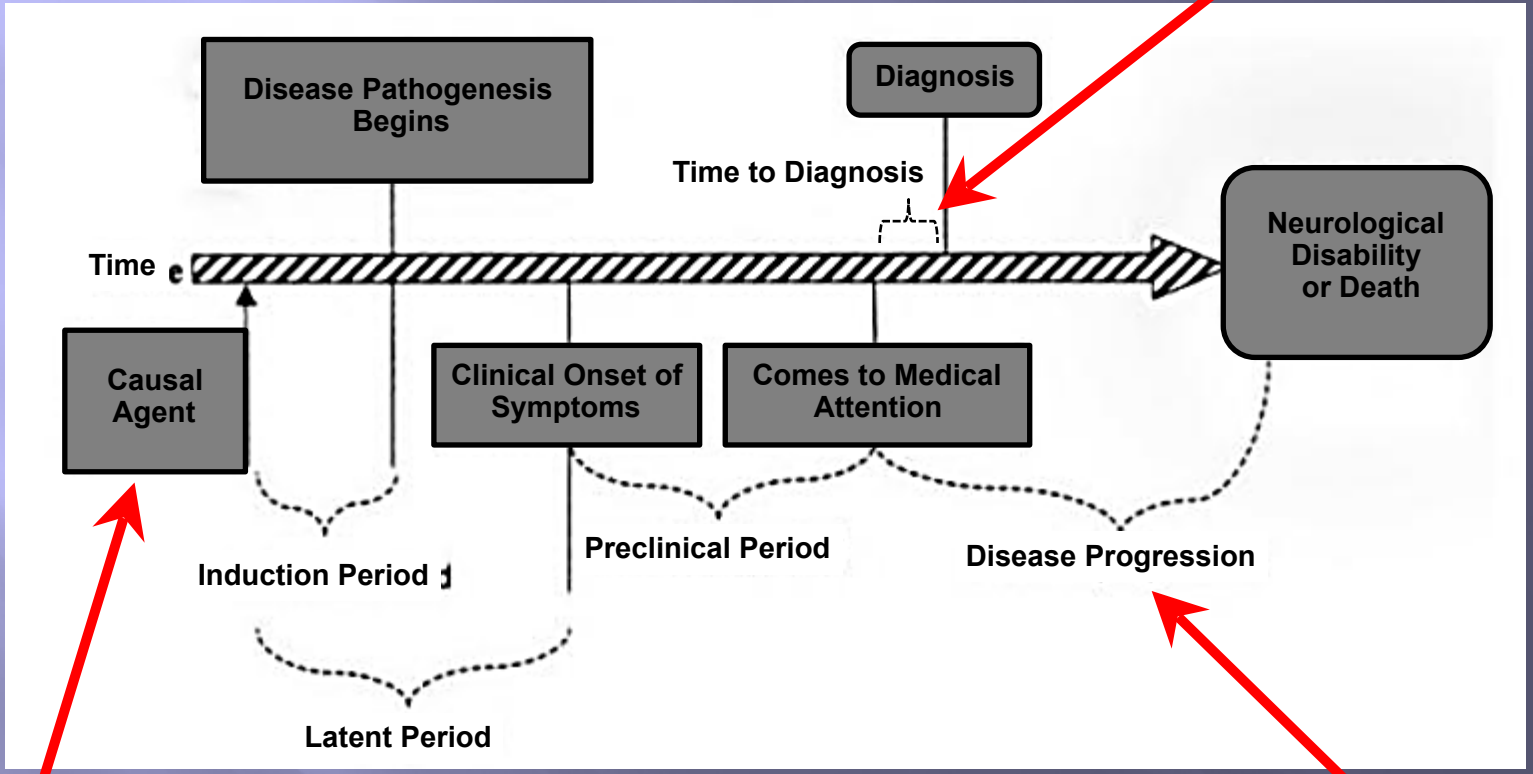
Rare Genes:
Estimated <1%
of Genetic Ataxia

Advanced Diagnostic: Clinical Exome Sequencing

If onset prior to age 20 years or suspected recessive inheritance consider simultaneous evaluation of parents.

Reasons to Diagnose Neurogenetic Disease

Can be decades (or never) for rare diseases



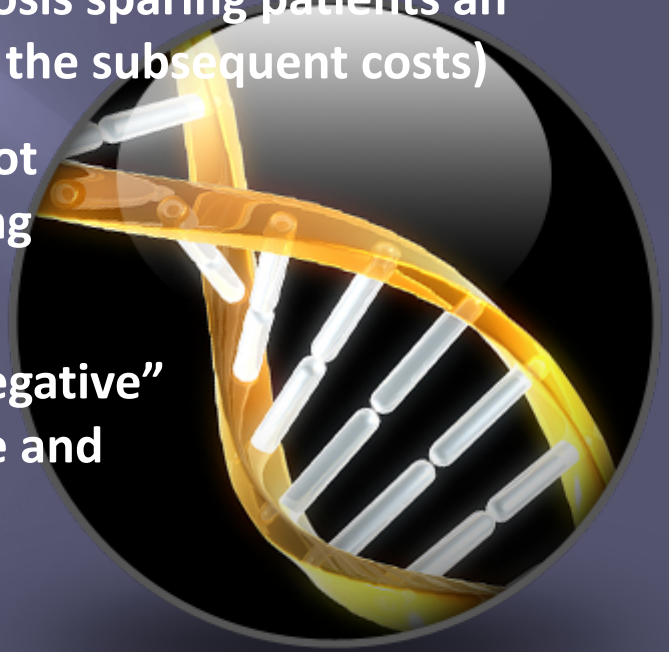
Establishing a genetic cause stops nonproductive search for other causes

- Disease or its comorbidities may be modifiable
- Genetic counseling

Modified from Nelson, Tanner, Van Den Eeden, McGuire eds: Neuroepidemiology, 2004

The ~~Future~~ Present of Clinical Genetic Testing

- Clinical Exome Sequencing is an unbiased form of genomic testing that assesses all 20,000 genes in the human genome simultaneously (cheap and efficient)
- Clinical Exome Sequencing improves diagnosis of clinically heterogeneous neurogenetic phenotypes (broad application)
- Clinical Exome Sequencing can lead to diagnoses that directly affect and improve patient management (clinically meaningful)
- Clinical Exome Sequencing reduces time to diagnosis sparing patients an extensive diagnostic odyssey (and sparing payers the subsequent costs)
- Clinical Exome Sequencing should compliment, not replace, a systematic patient evaluation (including high yield genetic testing if appropriate)
- Because results are not typically "positive" or "negative" physicians must receive training in the proper use and interpretation of clinical exome sequencing (disease-specific interpretation)



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Our Patients and their Families

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Questions?

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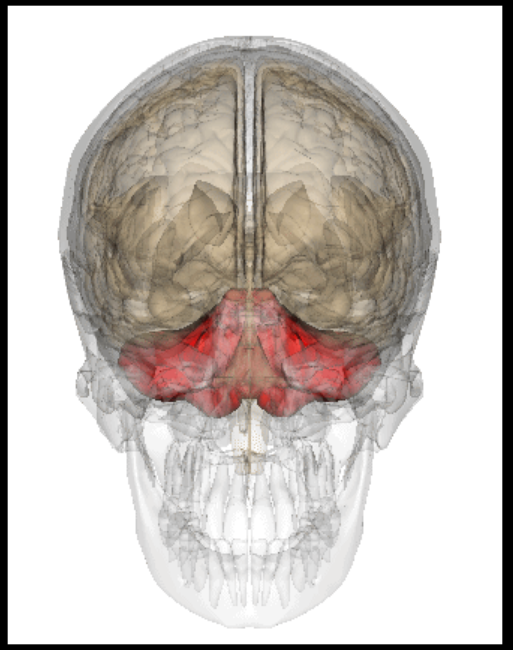

Image from Anatomography maintained by Life Science Databases(LSDB). From http://commons.wikimedia.org/wiki/File:Cerebellum_small.gif