Genetic testing for neurological diseases

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Service on advisory boards:

- Muscular Dystrophy Association
- Association Francaise contre les Myopathies
- Hereditary Disease Foundation
- Huntington's Disease Society of America
- Biogen Idec, Inc.
- Prosensa Therapeutics
- Summit Corporation

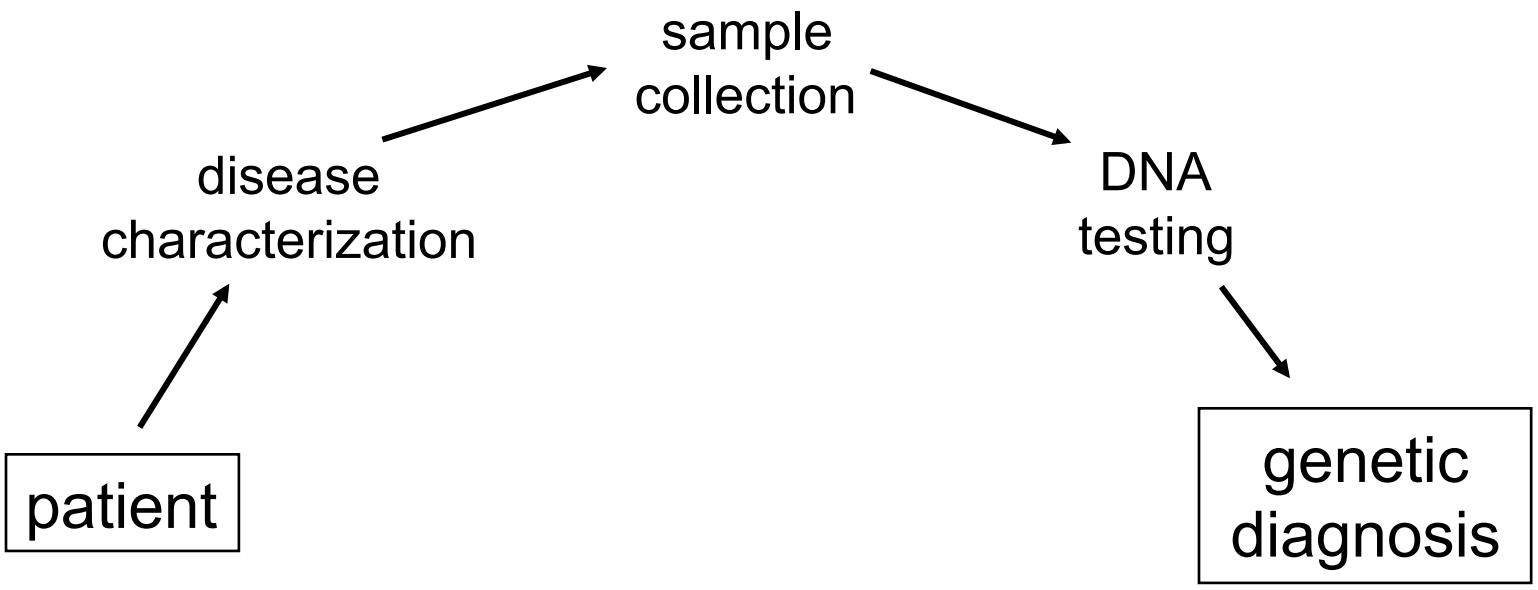
Sabbatical:

- Novartis Institutes for Biomedical Research: co-inventor on provisional patent application

Genetic testing for neurological diseases

- How it is done
  - Candidate gene testing
  - Gene panels
  - Genome-wide analysis
- Advantages
  - Disease-specific management & prognosis
  - Genetic counseling & testing for family members
- Risks
  - Pre-symptomatic testic
  - Incidental findings

Diagnosis of hereditary neurological diseases

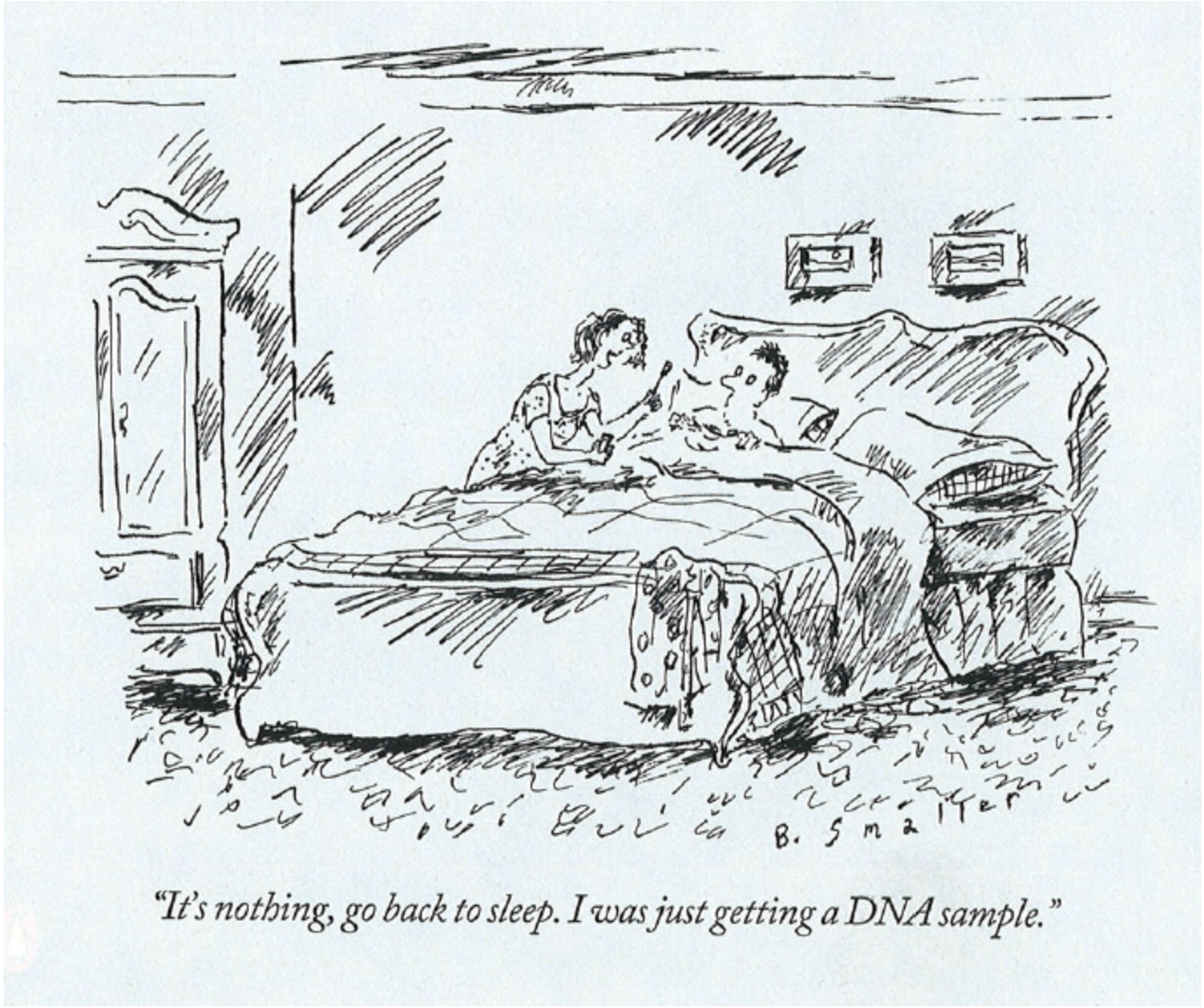


Disease characterization

- Neurological history & examination
- Family history
- Laboratory evaluation: relevant blood work (CK), electrophysiological tests (EMG/NCV), imaging
- Nerve or muscle biopsy if needed

Sample collection

- DNA can be extracted from white blood cells (EDTA tube), saliva, or tissue
- Stable at room temperature
- Very small amount needed
- Sampling of family members may be helpful



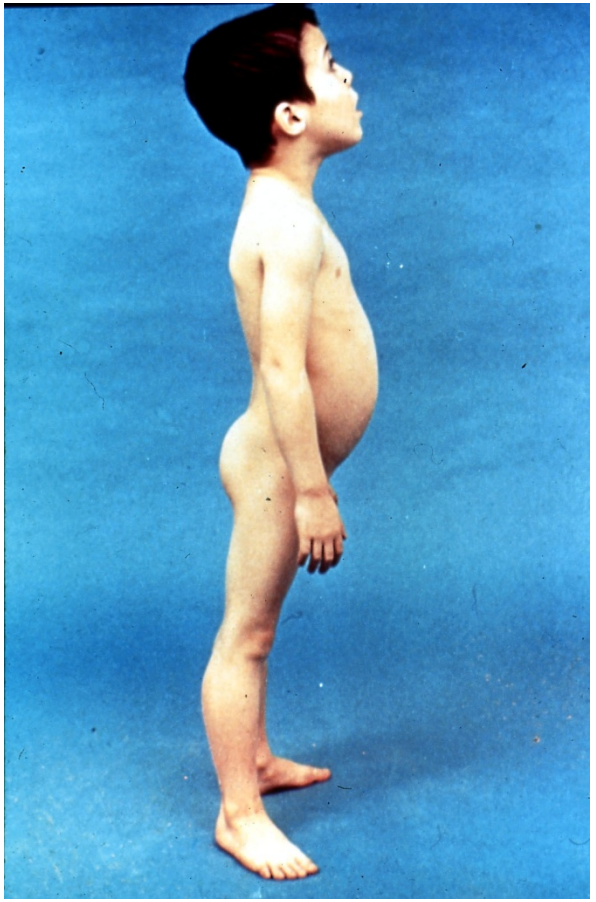
"It's nothing, go back to sleep. I was just getting a DNA sample."

Genetic testing laboratories

- NIH Genetic Testing Registry: www.ncbi.nlm.nih.gov/gtr
- Laboratories (examples):
  - Athena Diagnostics, Worcester, MA
  - PreventionGenetics, Marshfield, WI
  - GeneDX, Gaithersburg, MD
  - Medical Neurogenetics, Atlanta, GA
- Useful resources:
  - GeneReviews, Univ Washington
  - OMIM, Johns Hopkins Univ
  - Neuromuscular Disease website, Washington Univ, St. Louis

Candidate gene testing

Duchenne muscular dystrophy: X-linked recessive disease caused by mutation (usually deletion) in the gene for the muscle protein dystrophin.



Duchenne dystrophy diagnosis

- Characteristic clinical features: age of onset & distribution of weakness, X-linked recessive inheritance, high CK, EMG & biopsy myopathic (optional)
- Testing for deletion or duplication of one or more exons in the dystrophin gene: 75% positive
- Dystrophin gene sequencing for point mutations: additional 15% positive
- Dystrophin immunohistochemistry: ~100%

Duchenne muscular dystrophy

- Advantages of diagnosis:
  - Clinical management: steroid treatment; cardiac, pulmonary, & orthopedic support; physical & occupational therapy and assistive devices; etc.
  - Prognosis
  - Genetic counseling: carrier & prenatal testing
  - Enrollment in patient registries, clinical trials, support groups

Gene panels & genome-wide analysis

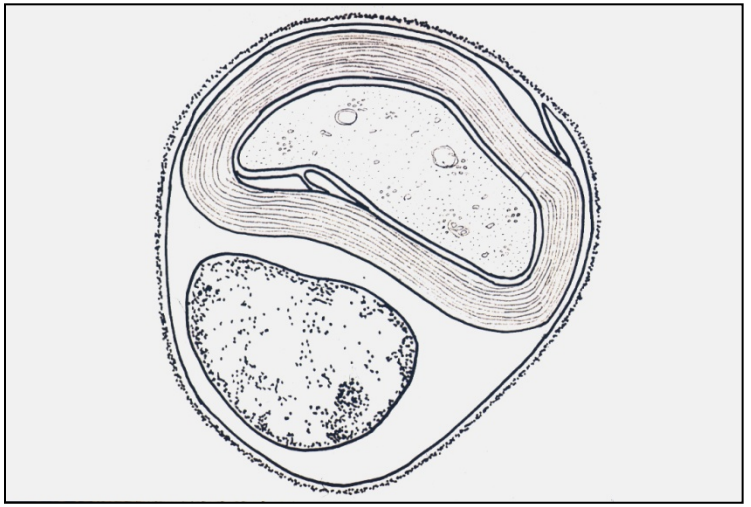
Charcot-Marie-Tooth disease:

- Progressive distal weakness and sensory loss
- Caused by degeneration of peripheral nerves
- Genetic heterogeneity: at least 78 different genes identified

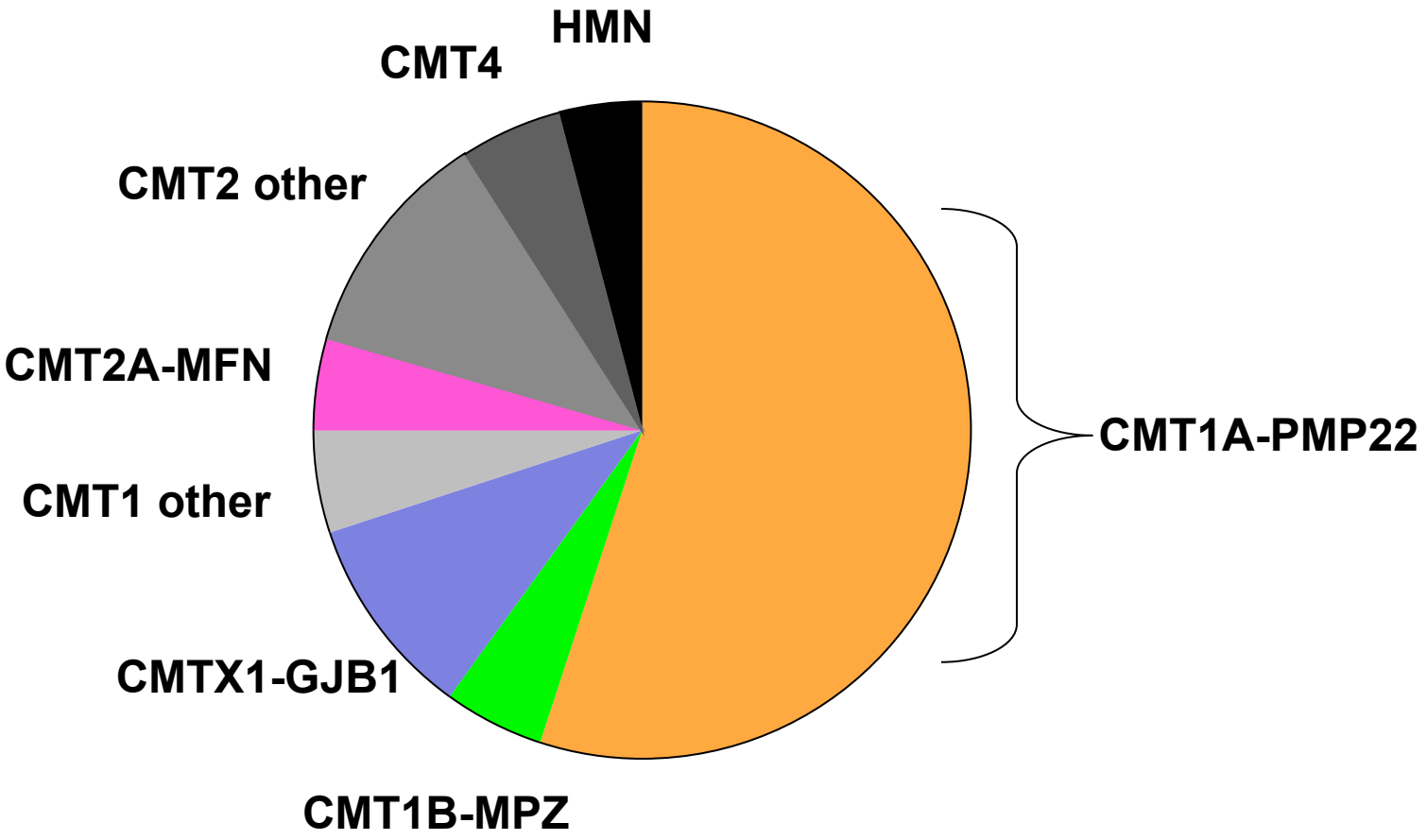
(from W. Allan)

Charcot-Marie-Tooth disease (CMT) : Two types

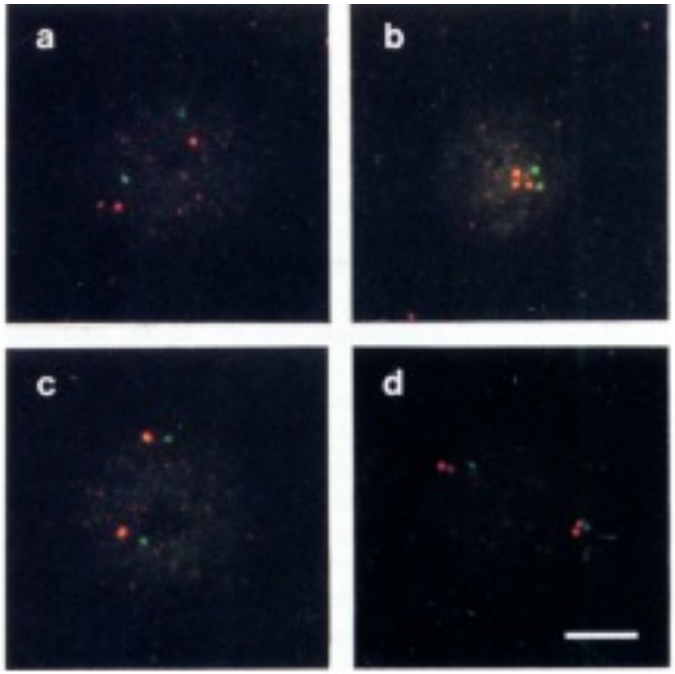
- CMT Type 1: loss of myelin
- CMT Type 2: axonal degeneration



CMT1A, 1B, X1, and 2A account for most hereditary neuropathy



Candidate gene testing: The most common cause of CMT1A is duplication of *PMP22*, which can be detected by fluorescent in situ hybridization (FISH):



– CMT1A
(red = signal for *PMP22* region)

Normal pattern

(Lupski et al, 1991)

Genetic diagnosis of other CMTs is by DNA sequencing.

CMT diagnosis by DNA sequencing

- CMT gene panels:
  - CMT type 1
  - CMT type 2
  - all CMTs
- Genome-wide analysis

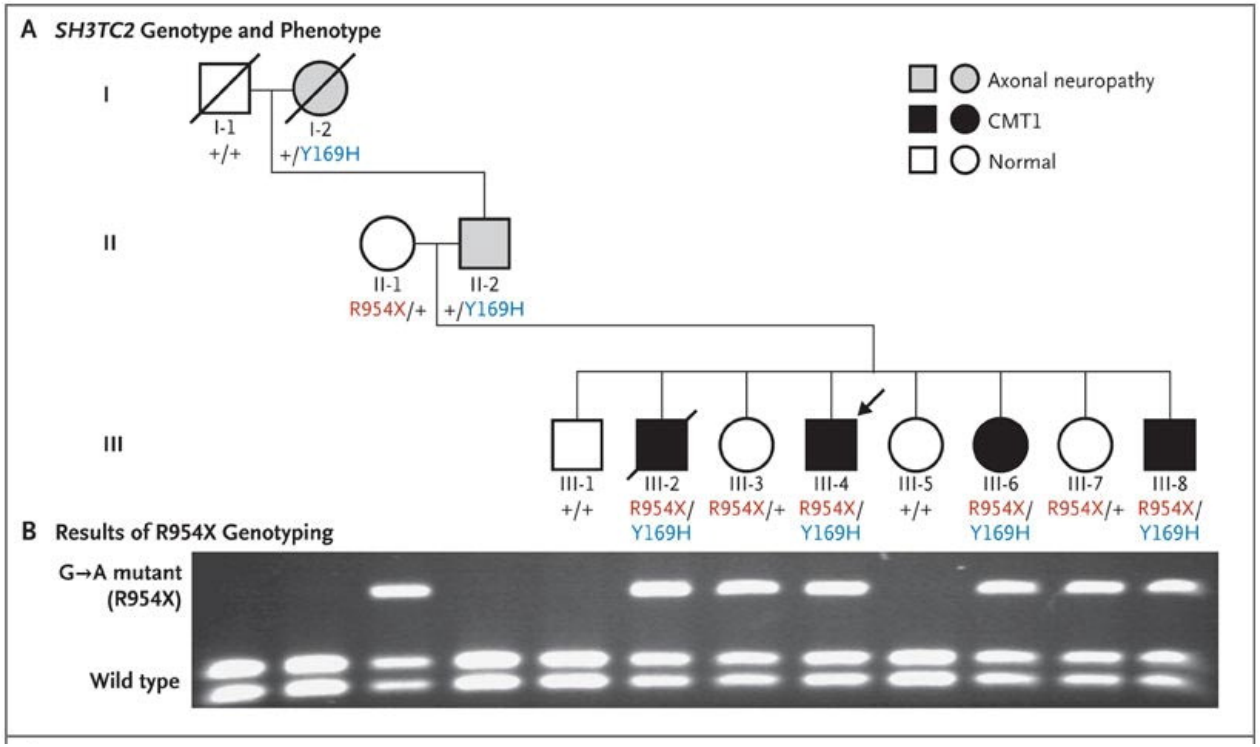
CMTX4 (Cowchock syndrome): *AIFM1*

- severe X-linked recessive axonal neuropathy with deafness and cognitive impairment
- single family mapped to Xq24-q26
- mutation found in mito-chondrial apoptosis inducing factor, AIFM1

Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy.

Lupski JR, Reid JG, Gonzaga-Jauregui C, et al.

N Engl J Med. 2010



Genome-wide analysis

- Whole genome vs. exome sequencing
- Confirming pathogenicity of sequence variants:
  - Known association with disease
  - Segregation in family
  - Not present in control databases (e.g., NHLBI)
  - Cross-species conservation
  - Effect on protein structure & function

Repeat expansion diseases

- At least 30 diseases are known to be caused by expansion of simple sequence repeats, most commonly trinucleotide repeats.
- Nearly all these disorders have neurological manifestations.
- In many cases the expanded repeats are unstable and increase in length from one generation to the next, resulting in increased disease severity (anticipation).

Huntington's disease

- Autosomal dominant neurodegenerative disease
- Characterized by progressive chorea, psychological changes, and cognitive decline
- Loss of neurons most prominent in the striatum
- Caused by expansion of a CAG repeat in the *huntingtin* gene on chromosome 4p

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Huntington's disease

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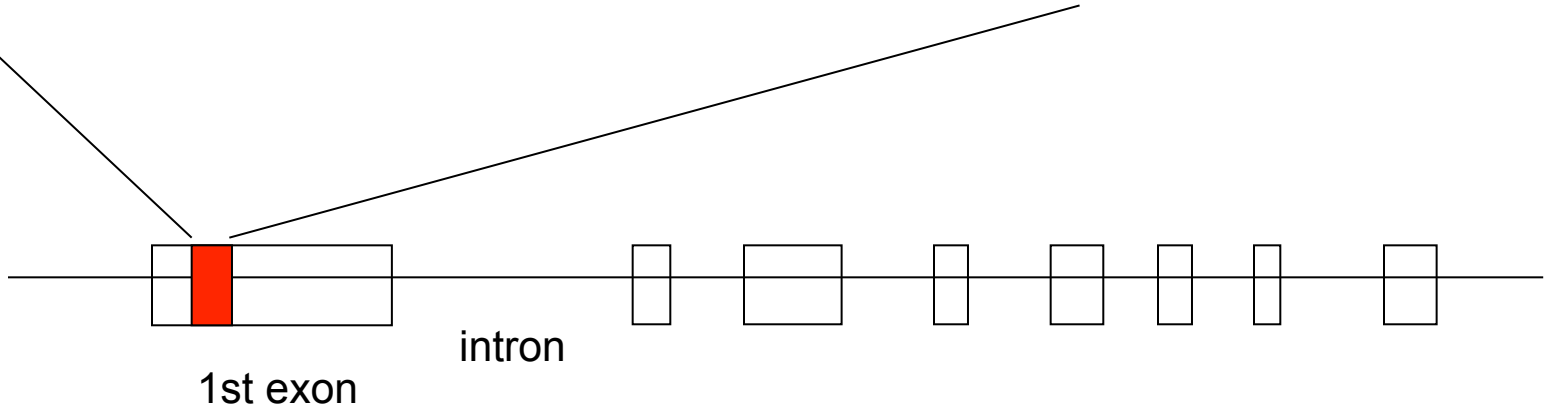
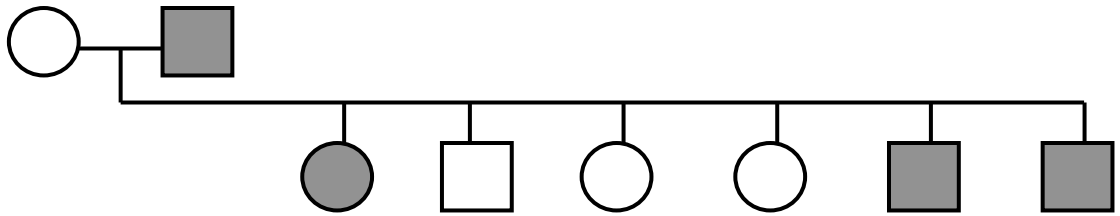
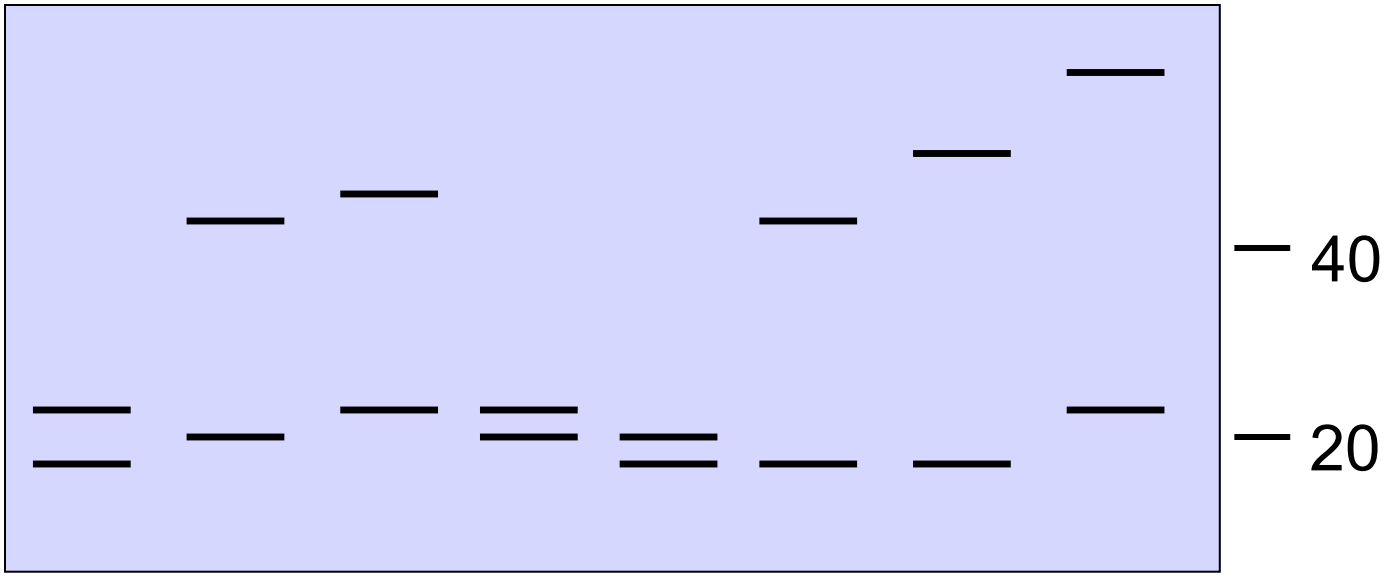
Normal

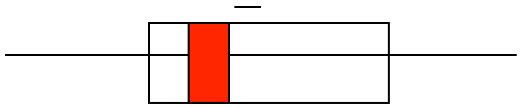


Huntingtin gene

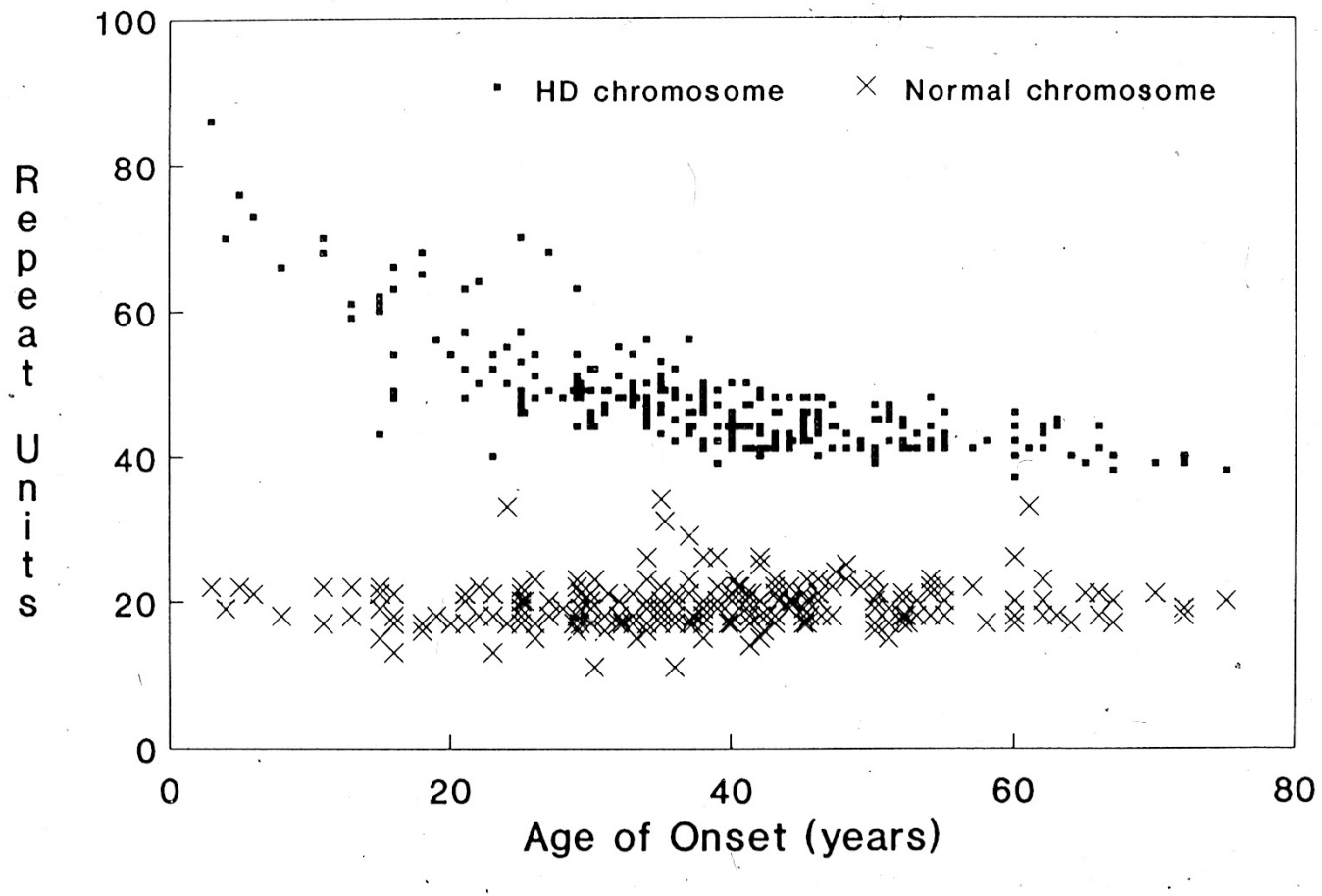




Analysis of repeat length by polymerase chain reaction (PCR)



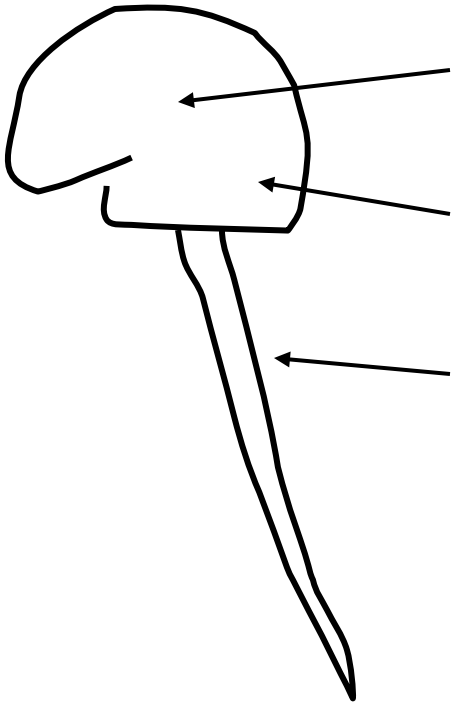
Huntington's disease: correlation of repeat length with age of onset



Huntington's disease

- Because of psychosocial risks, it is important to proceed carefully with genetic testing of asymptomatic individuals at risk for Huntington's disease, with counseling before and after testing.
- Similar considerations apply to other late onset neurodegenerative disorders.

Polyglutamine disorders



- Huntington's disease
- Spinocerebellar ataxias
- Kennedy's disease

- Other late onset neurodegenerative diseases with known genes: Alzheimer's, Parkinson's, ALS

Incidental findings

- Genome-wide analysis may uncover unexpected incidental mutations.
- Some of these findings may have therapeutic implications, e.g., breast & colon cancer gene mutations.
- ACMG has published a list of 56 genes where mutations should be reported to the patient (found in ~2-3% of exomes sequenced).

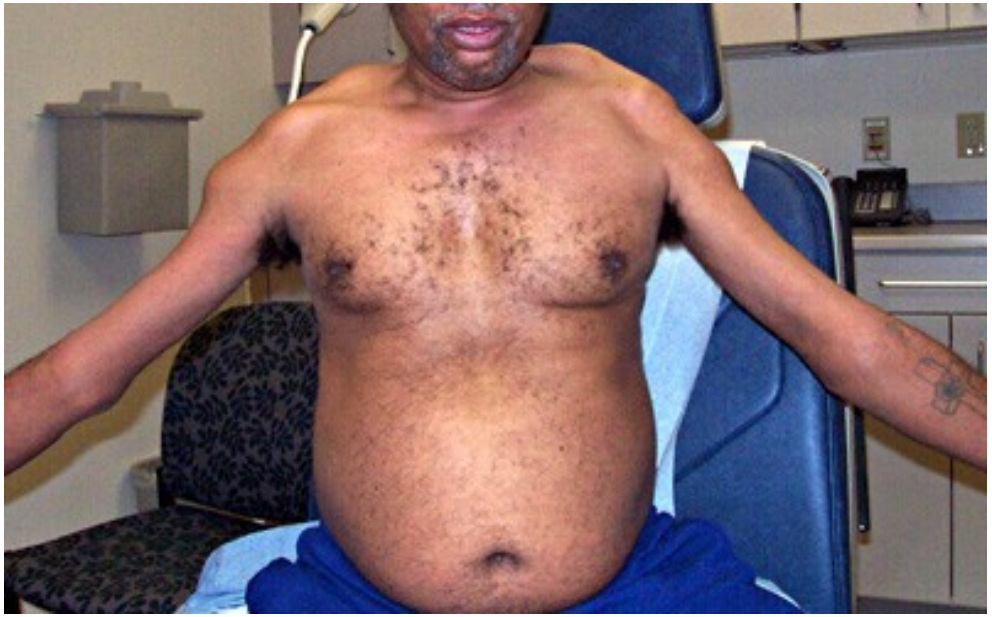
Case history

A 17 year old girl complained of progressive difficulty walking. At age 5, the right foot was internally rotated. Age 7, exam showed mild weakness of the arms and right leg and deformity of the right foot. Head and spine MRI were normal. EMG showed myopathic units in the peroneus longus and biceps muscles.

Case history (cont.)

Age 10, braces required for progressive deformity of the feet & frequent falls. She also had difficulty throwing a ball. Exam showed a transverse smile, normal muscle tone, right > left winged scapulae, proximal weakness of the arms, bilateral foot deformities, normal sensory examination, and diffuse hyporeflexia. Muscle biopsy was normal. She was given a diagnosis of facioscapulohumeral dystrophy.

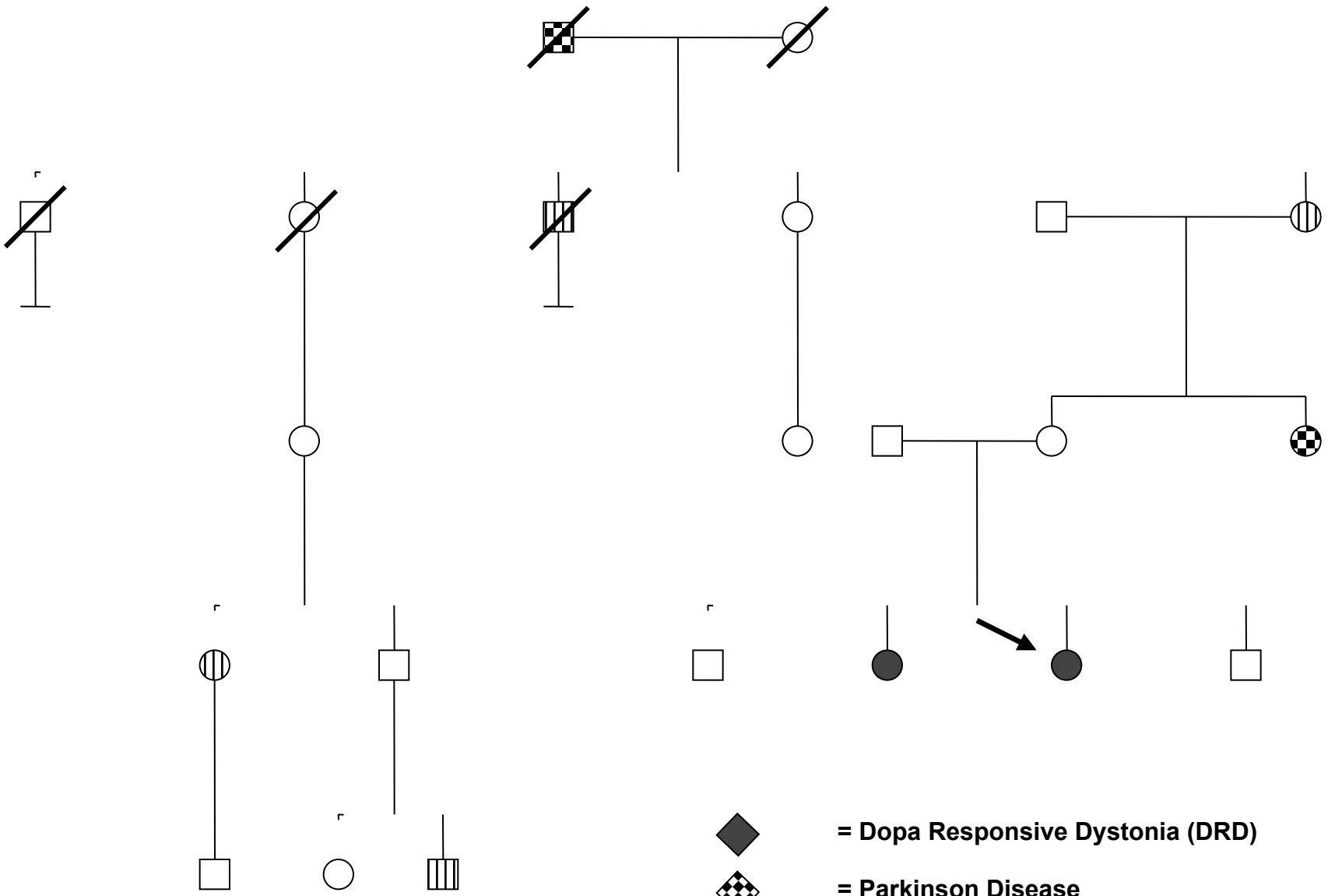
Facioscupulohumeral muscular dystrophy



Case history (cont.)

Age 15, although she could still walk the short distance from the car to school in the morning, she could no longer walk that distance by the end of the day. At dinner she had difficulty raising her head to eat and was extremely slow to complete her meal. She used a wheelchair for all but short distances. She began to have episodes where her legs “stiffened up” and “locked.”

A diagnostic test was performed ...


= Dopa Responsive Dystonia (DRD)
= Parkinson Disease
= Neurological symptoms consistent w/ DRD

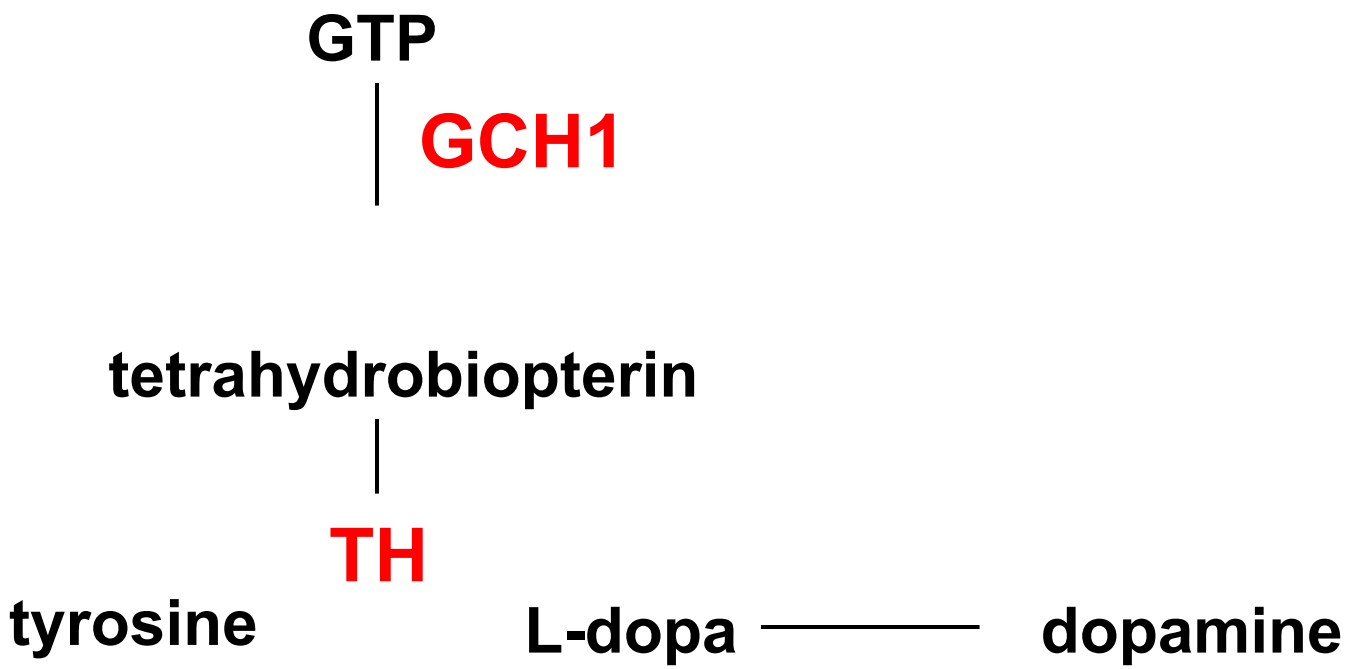
Dopa responsive dystonia

- Childhood onset of dystonia involving one or both legs (other presentations: focal dystonia, parkinsonism, spastic paraparesis, “myopathy”).
- Diurnal variation in tone.
- Therapeutic response to low dose levodopa.

Dopa responsive dystonia: genetics

- Mutations in two genes that encode enzymes required for dopamine synthesis:
  - GTP cyclohydrolase 1 (GCH1): autosomal dominant, 85 different mutations [gly108asp in this family]
  - Tyrosine hydroxylase (TH): autosomal recessive, 10 different mutations

GCH1 and TH are important enzymes in dopamine synthesis



Dopa-responsive dystonia is important to diagnose as a treatable hereditary neurological disease.

Take home lessons

- Genetic testing is rapidly evolving as a diagnostic tool.
- This allows comprehensive diagnosis of hereditary neurological diseases, with important implications for clinical management.
- Pre-symptomatic diagnosis should be done with care.
- Incidental findings arise from genome-wide analysis, and a strategy is needed for dealing with them.

The which observed, a man may prophesy,

With a near aim, of the main chance of things

As yet not come to life, which in their seeds

And weak beginnings lie intreasured.

Henry IV, Pt II