MOLECULAR DIAGNOSTICS PATERNITY TESTING

Molecular diagnostics

- collection of techniques used to analyse biological markers in the genome and proteome by applying molecular biology to medical testing

- techniques are used to:
- diagnose and monitor disease
- detect risk
- decide which therapy will work best for individual patient

- by analysing the specifics of the patients and their disease, molecular diagnostics offers the prospect of personalised medicine

Molecular diagnostics

- these tests are useful in a range of medical specialisms, including:

oncology - tumor markers

neurology - antigens and antibodies associated with neurodegenerative diseases

pulmonary medicine – cystic fibrosis

metabolic diseases - phenylketonuria

immunology – markers associated with autoimmune diseases

infectious disease – chlamydia, influenza virus and tuberculosis

prenatal – dynamics of markers in physiological pregnancy and their changes in pathological pregnancy

Molecular diagnostics

Marker = laboratory demonstrable signs, which disease manifests itself, or from which it is derived

Requirements:

- associated with disease
- determinable in blood, urine or tissue
- screening
- designed to refine the diagnosis, monitoring of therapy

humoral – located in body fluids

cellular – receptors on cells, presence of specific proteins in cell membrane or inside cells

genetics – in DNA or RNA

Molecular diagnostics - strategy

Direct DNA/RNA diagnostics

- determine whether DNA carries a mutation or not
- detection of mutation **in genes** associated with disease

Indirect DNA/RNA diagnostics

- use of binding markers in family studies
- reveals allele-related illness in the family
- use of **polymorphic sites** of the human genom
- today is not used (only for paternity testing)

Diagnostics of proteins and their description

- whether the protein linked with disease is present or not
- changes in conformation of physiologic proteins
- presence of receptors

Molecular diagnostics - methods

Direct DNA/RNA diagnostics

- detection of causal mutations in responsible gene always confirm clinical diagnosis (ADVANTAGE)

we must know: - gene to be analyzed
- standard (wild type) gene sequence

2 procedures: - detection of well known mutations (scoring)
- search of unknown mutations (scanning)

PCR

SSCP on gel (single-strand conformation polymorphism)

- changes in DNA primary structure change also secondary structure and it changes the mobility of DNA on the gel

DGGE (denaturing gradient gel electrophoresis)

- changes in structure influence the speed of denaturing

Molecular diagnostics - methods

Indirect DNA/RNA diagnostics
- paternity testing

Diagnostics of proteins and their description
We must know: - protein to be analyzed
- physiological levels in organism
- its structure

ELISA (enzyme-linked immunosorbent assay)
- detection of antigen (protein), specific antibodies

Western blot - electrophoretic separation, than detection of specific proteins on membrane

2D electrophoresis

Crystallography, NMR, MS-MALDI – determining of structure

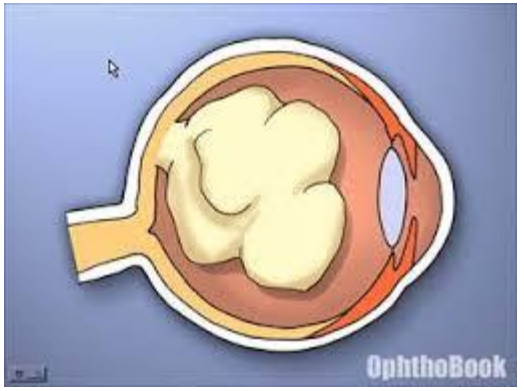
Molecular genetics markers of selected diseases

Oncology

Retinoblastoma

- 3 % of tumors in children up to 15 years
- malignant tumor that rapidly develops from the immature cells of a retina
- most common primary malignant intraocular cancer in children, and it is almost exclusively found in young children
- AD heredity or sporadically

Causes: - mutation in Rb1 gene
- „brake“ of cell cycle
- tumor suppressor gene



Molecular genetics markers of selected diseases

Oncology

Retinoblastoma

Diagnostics:

red reflex - checking for a normal reddish-orange reflection from the eye's retina with an ophthalmoscope or retinoscope from approximately 30 cm / 1 foot, usually done in a dimly lit or dark room

RT PCR - is a laboratory technique combining reverse transcription of RNA into DNA (in this context called complementary DNA or cDNA) and amplification of specific DNA targets using polymerase chain reaction (PCR)

Molecular genetics markers of selected diseases

Oncology

Breast carcinoma

- 16 % of women tumors, but 1 % of patients are men
- malignant tumor of mammary gland
- AD heridity or sporadically

symptoms:

- area of thickened tissue in the breast, or a lump in the breast
- pain in the breast that does not change with the monthly cycle
- pitting or redness of the skin of the breast
- rash around or on one of the nipples
- sunken or inverted nipple
- change in the size or shape of the breast

Molecular genetics markers of selected diseases

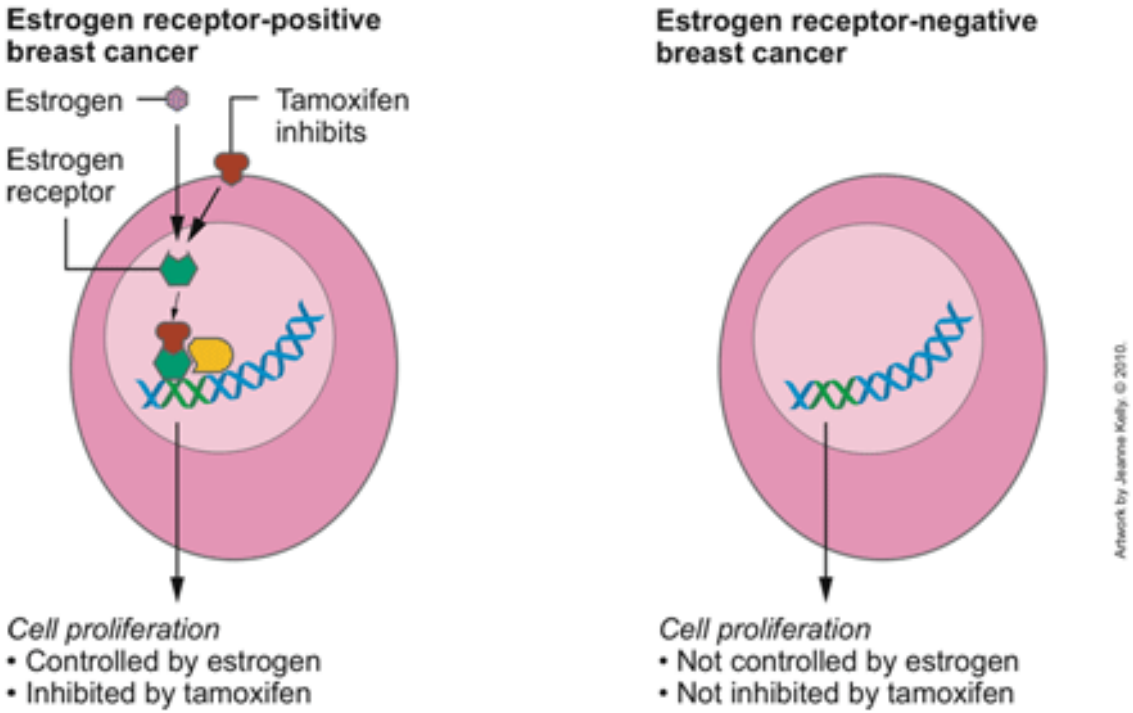
Oncology

Breast carcinoma

Causes: - mutation in BRCA1, BRCA2 gene
- receptor HER2/neu
- estrogen receptor

Diagnostics: self-monitoring
mammograms
FISH, PCR

Estrogen Receptor-Negative Breast Cancer



Molecular genetics markers of selected diseases

Neurology

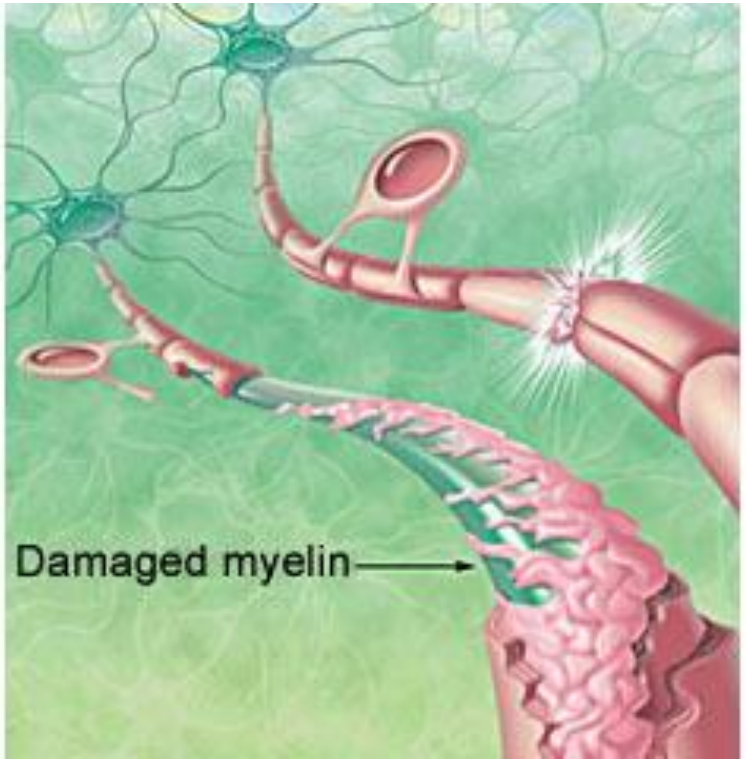
Sclerosis multiplex

- serious neurodegenerative disease
- affects especially girls from 17 to 30 years
- is a **demyelinating disease** in which the insulating covers of nerve cells in the brain and spinal cord are damaged
- this damage disrupts the ability of parts of the nervous system to communicate, resulting in a range of signs and symptoms, including physical, mental, and sometimes psychiatric problems

Central nervous system
(brain and spinal cord)

In multiple sclerosis the myelin sheath, which is a single cell whose membrane wraps around the axon, is destroyed with inflammation and scarring

A.D.A.M.



Molecular genetics markers of selected diseases

Neurology

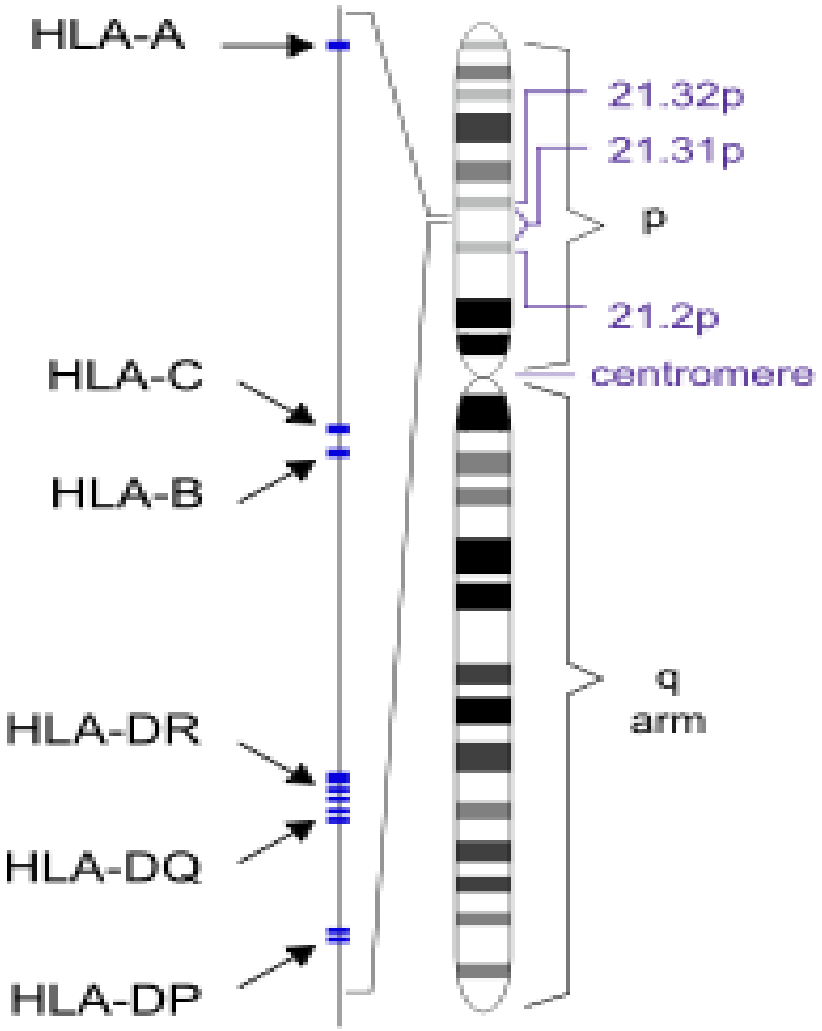
Sclerosis multiplex

- is not considered a hereditary disease; however, a number of genetic variations have been shown to increase the risk
- some of these genes appear to have higher levels of expression in microglial cells

Causes:

- changes in **human leukocyte antigen** (HLA) system
- group of genes on chromosome 6 that serves as the **major histocompatibility complex** (MHC)
- mutation in genes IL2RA a IL7RA (receptors for interleukins 2 a 7)

HLA
MHC Complex



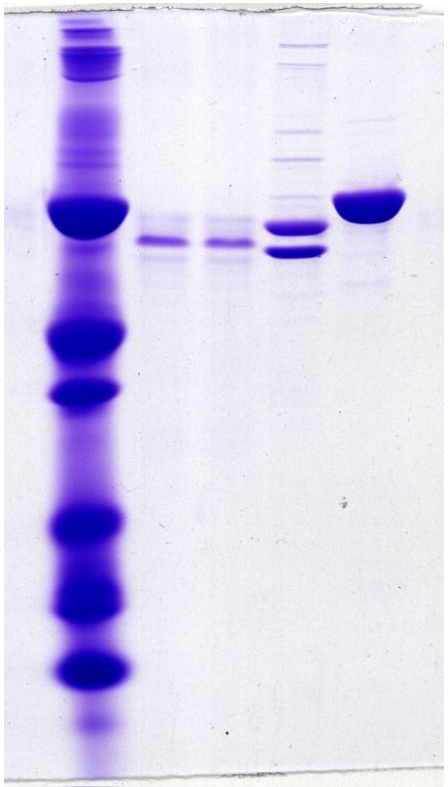
human chromosome 6

Molecular genetics markers of selected diseases

Neurology

Sclerosis multiplex

Diagnostics: - levels of specific antibodies in cerebro-spinal fluid
- cerebrospinal fluid is tested for oligoclonal bands of IgG on electrophoresis
- MR



Molecular genetics markers of selected diseases

Neurology

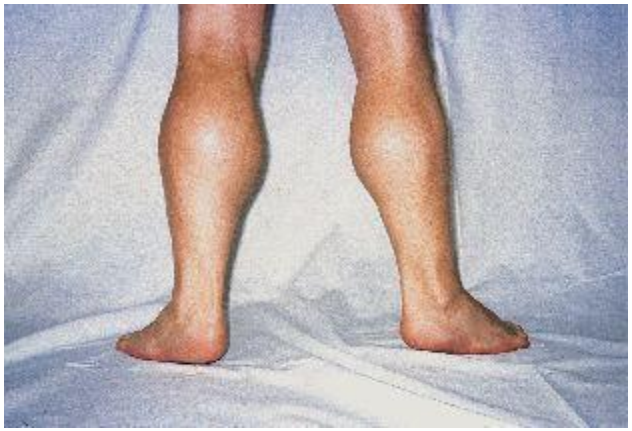
Duchenn muscular dystrophy

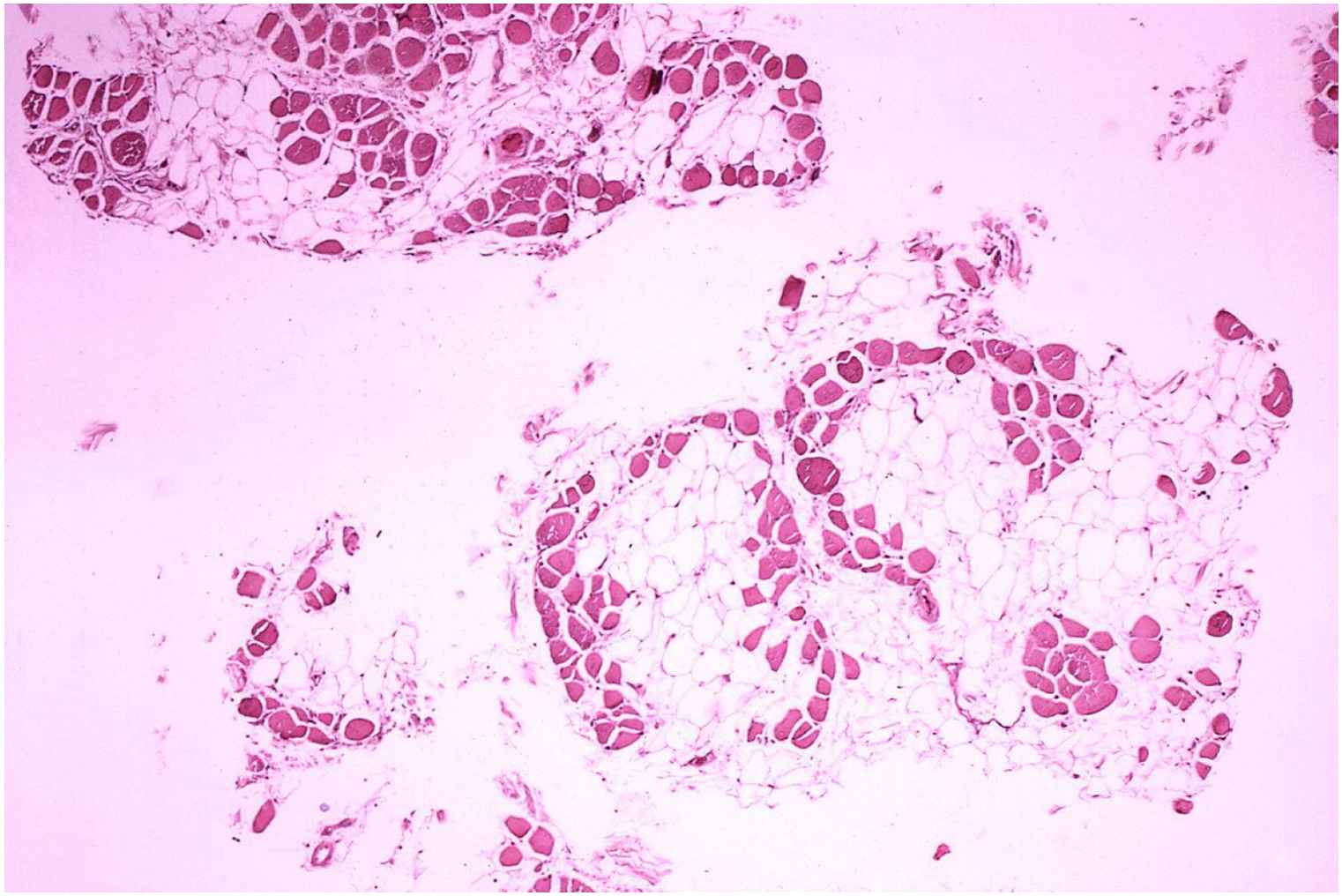
- serious neurodegenerative disease, first signs between 3-6 years, changes in posture, walking on tiptoe

symptoms:

- muscle weakness, frequent falls, fatigue, difficulty with motor skills (running, hopping, jumping)
- enlarged calf muscles (due to increased fat content)
- in 13 years invalidity (wheelchair)
- expectancy 20 years

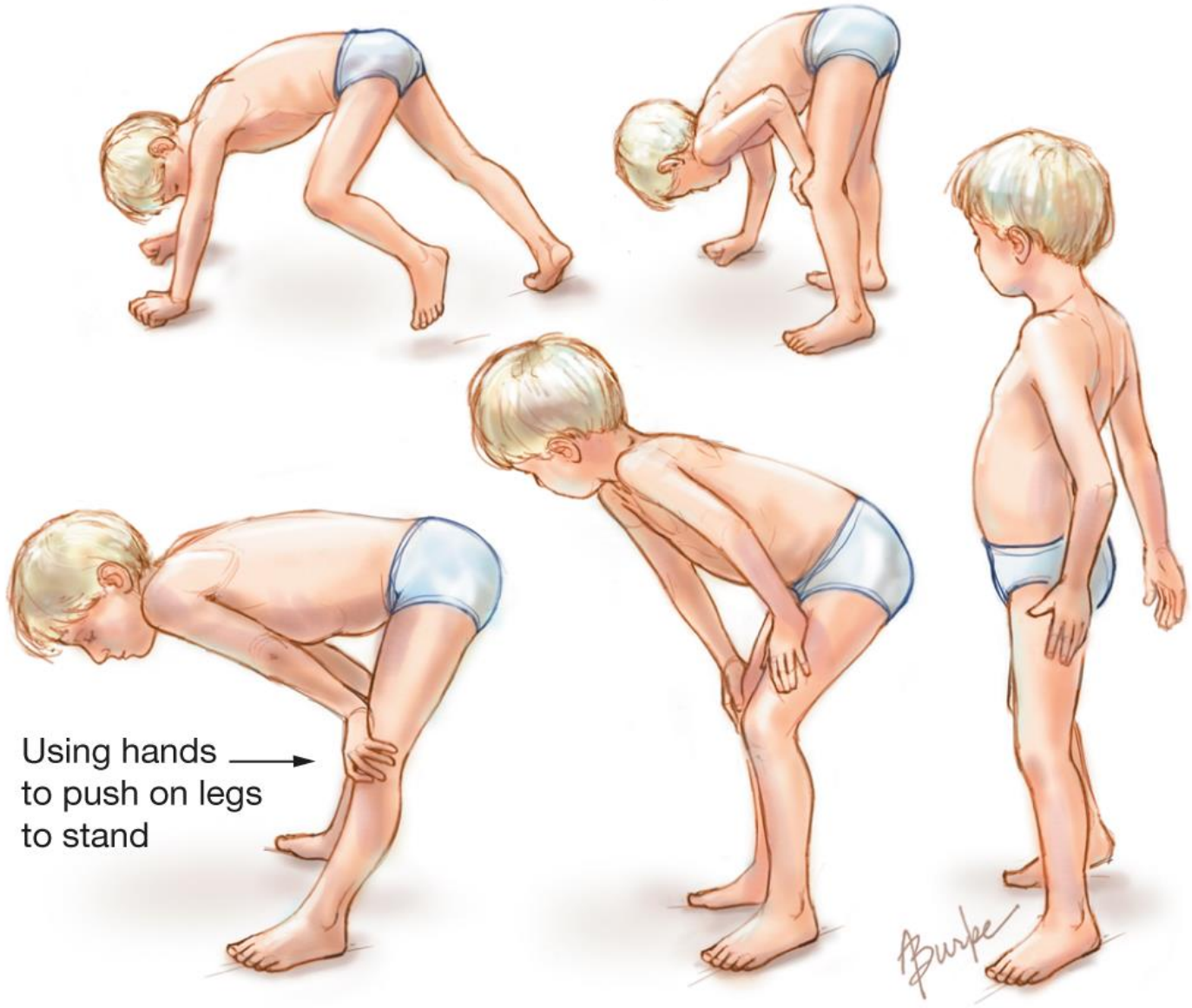
lighter form: Becker muscular dystrophy

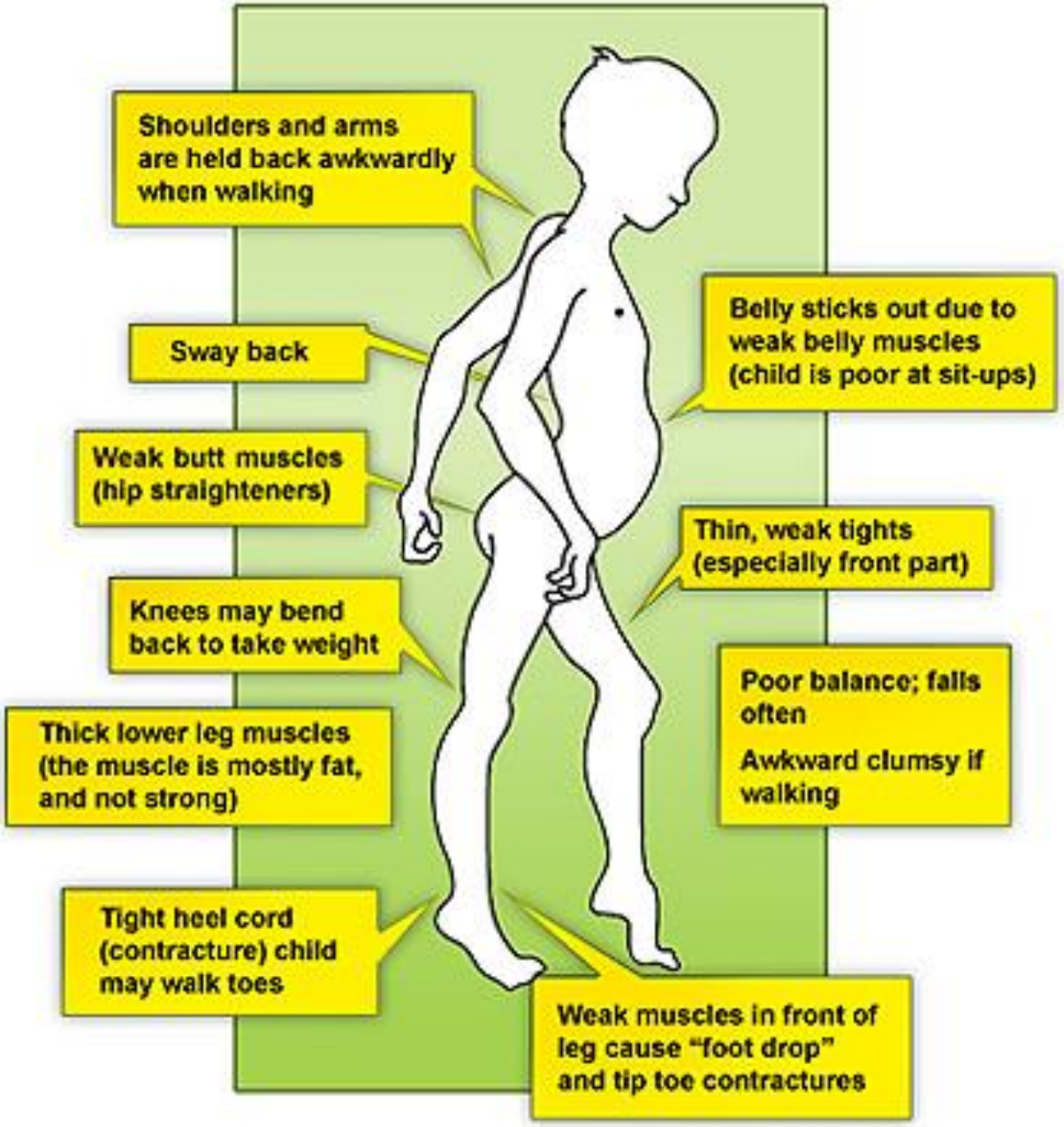




cross section of the calf muscle shows extensive replacement of muscle fibers by fat cells

Gowers Sign




Shoulders and arms are held back awkwardly when walking
Sway back
Weak butt muscles (hip straighteners)
Knees may bend back to take weight
Thick lower leg muscles (the muscle is mostly fat, and not strong)
Tight heel cord (contracture) child may walk toes
Belly sticks out due to weak belly muscles (child is poor at sit-ups)
Thin, weak tights (especially front part)
Poor balance; falls often
Awkward clumsy if walking
Weak muscles in front of leg cause "foot drop" and tip toe contractures

Molecular genetics markers of selected diseases

Neurology

Duchenn muscular dystrophy

Cause: - body can´t product muscle protein dystrophin (in correct form)
(necessary for proper functioning of muscles)
- gene for dystrophin has 79 exons,
70 % frameshift, 30 % mutation *de novo*
- Xp21

Diagnostics: - **FISH**
- **MLPA** (multiplex ligation-dependent probe amplification)

Molecular genetics markers of selected diseases

Neurology

Duchenn muscular dystrophy

Treatment: - **exon skipping** (antisense oligonucleotides)
- structural analogs of DNA
- allow faulty parts of the dystrophin gene to be skipped when it is transcribed to RNA for protein production, permitting a shorter but more functional version of the protein to be produced

- **stem cells replacement**

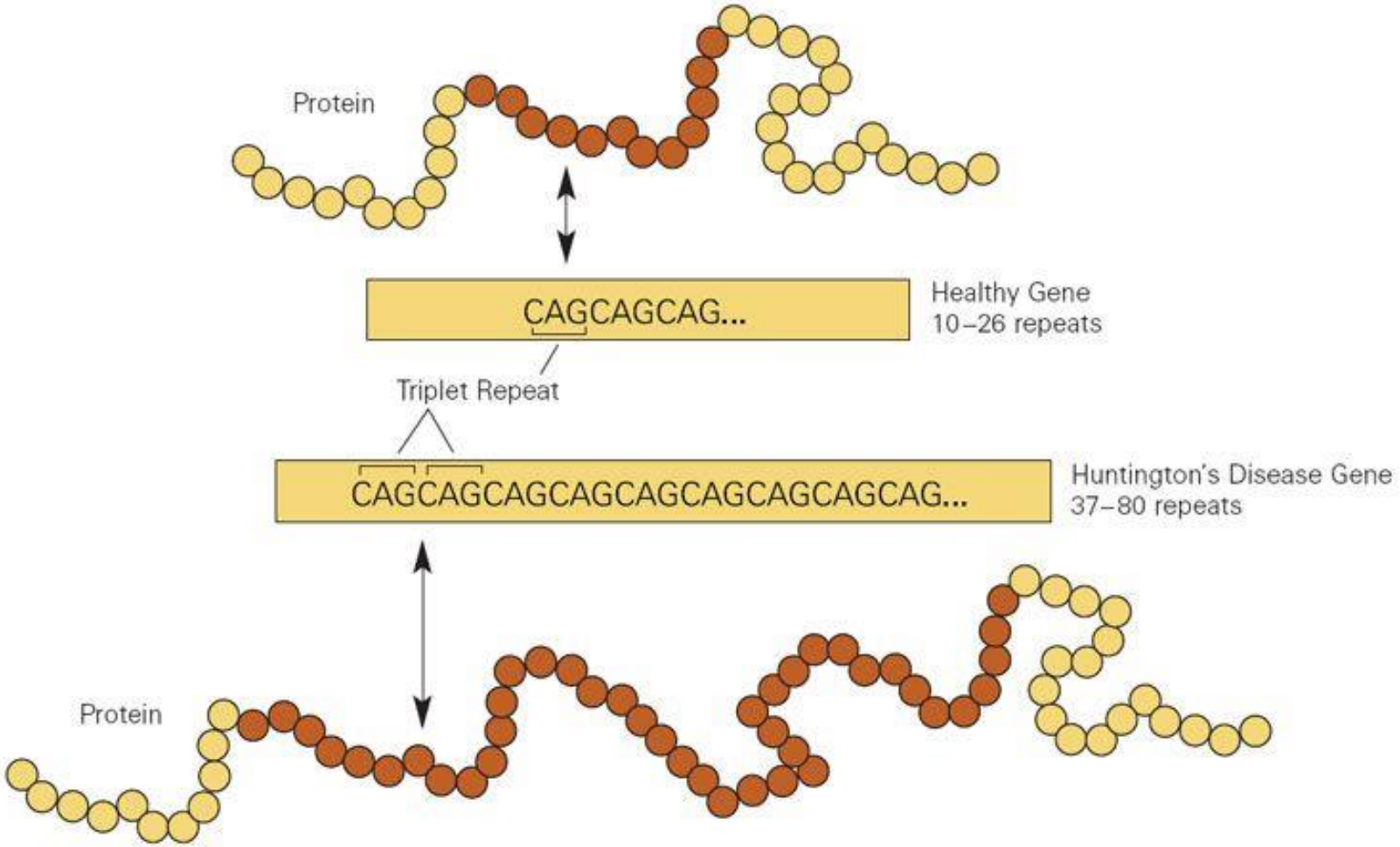
Molecular genetics markers of selected diseases

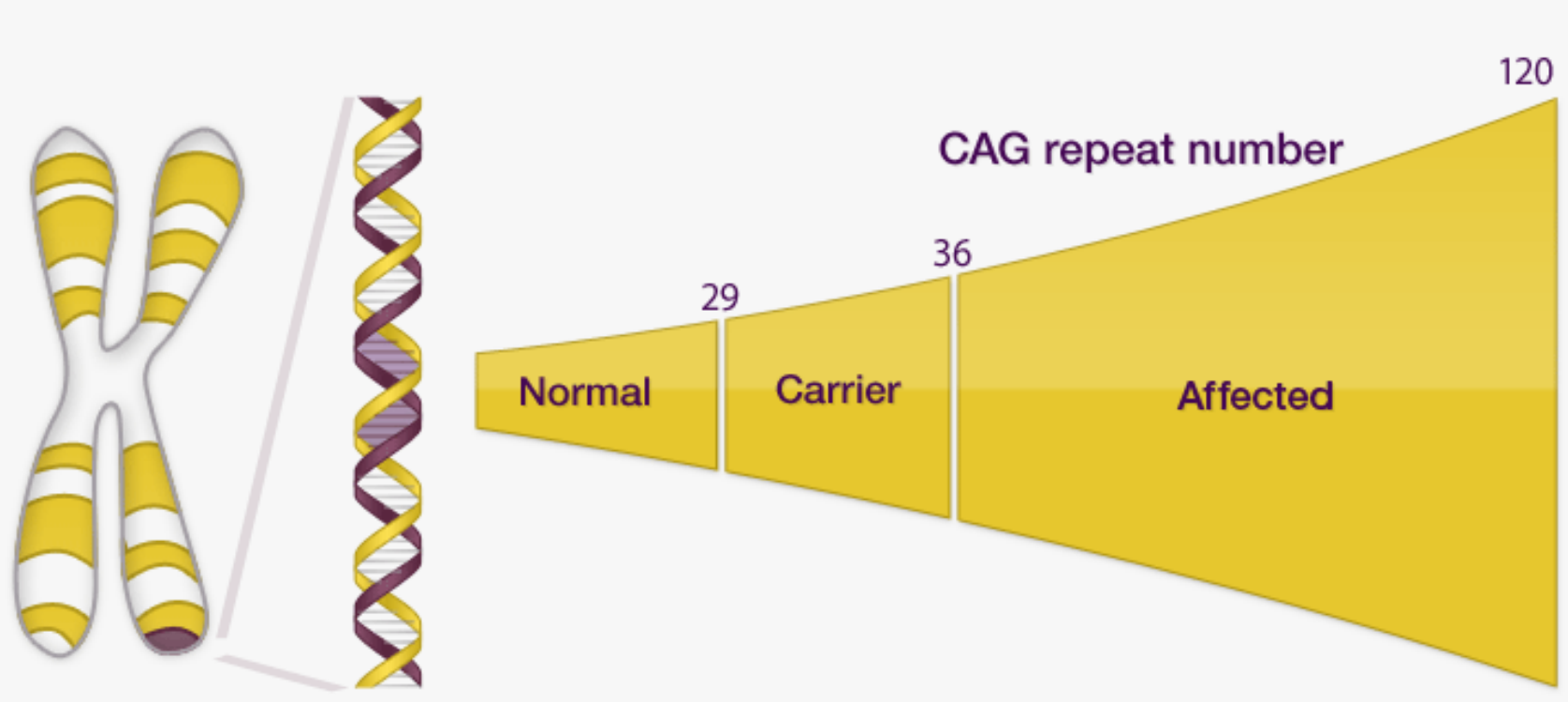
Neurology

Huntington disease
- serious neurodegenerative disease, AD heridity
- affects between 40 and 50 years
- problems with mobility, leads to dementia

Causes: - **expansion of trinucleotides repeats**
- gene for huntingtin, protein accumulates in brain and physiological enzymes are not able to degrade it

Diagnostics: **triplet primed PCR**


Protein
CAGCAGCAG...
Healthy Gene
10–26 repeats
Triplet Repeat
CAGCAGCAGCAGCAGCAGCAGCAGCAG...
Huntington's Disease Gene
37–80 repeats
Protein


120
CAG repeat number
36
29
Normal
Carrier
Affected

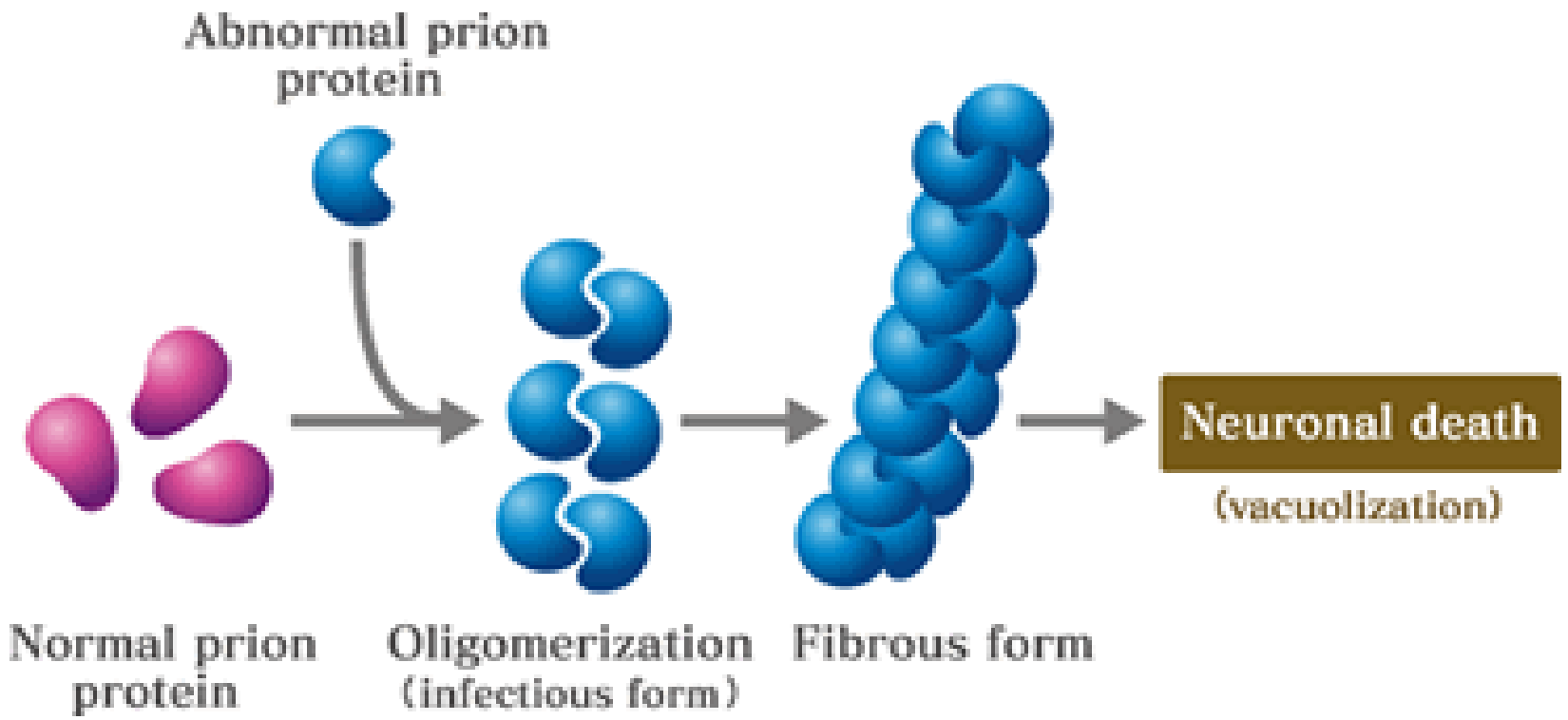
Molecular genetics markers of selected diseases

Neurology

Creutzfeldt-Jakob disease
- neurodegenerative disease, spongiform encephalopathy
- familial, sporadic or iatrogenic
- long incubation period, affected is **phenotype**

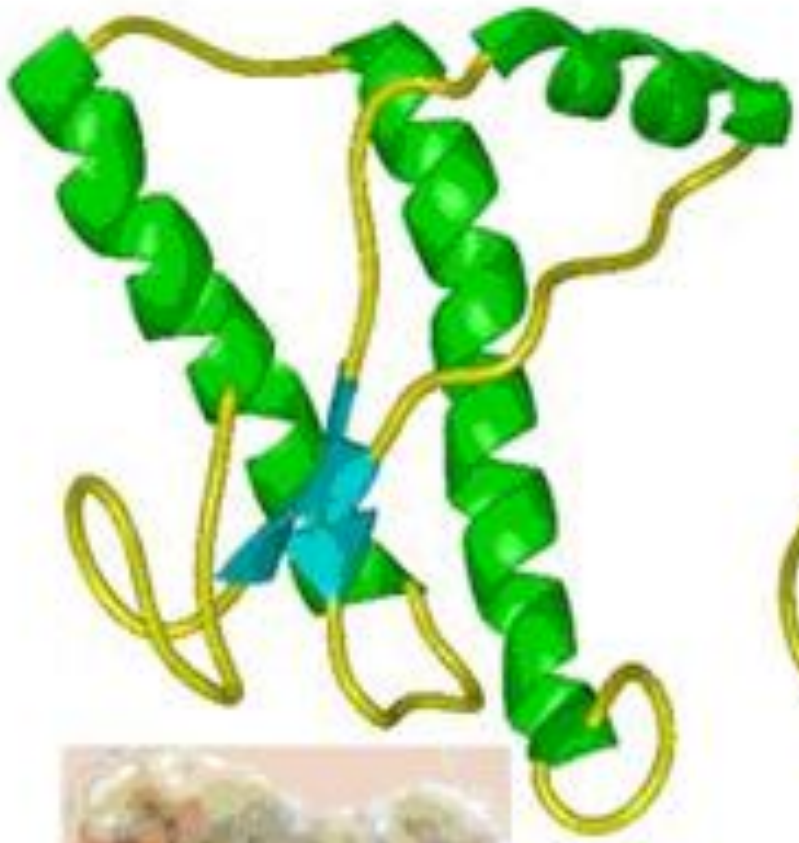
Causes: - prion (infectious protein particle, PrP^{Sc}) changes the structure of standard prion protein (PrP^{C}) to pathological isoform (PrP^{Sc}), which accumulates in brain and physiological enzymes can´t degrade it

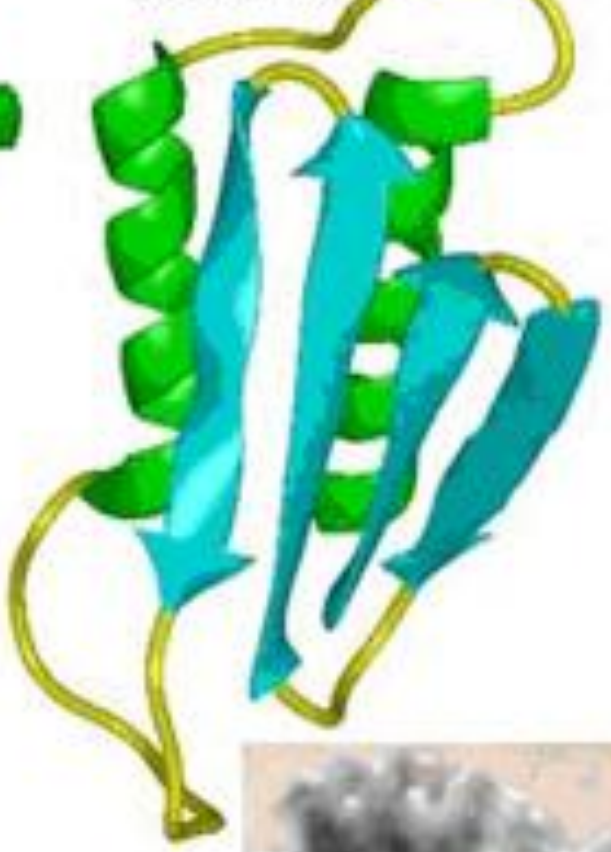
Diagnostics: **Western blot**
ELISA
immunohistology – direct detection of PrP^{Sc} in brain tissue
neurohistopathology

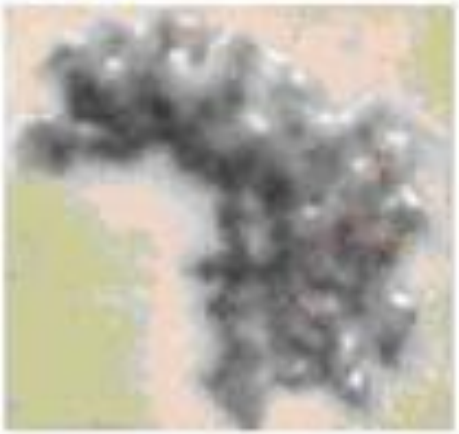

Abnormal prion protein
Neuronal death
(vacuolization)
Normal prion protein
Oligomerization (infectious form)
Fibrous form

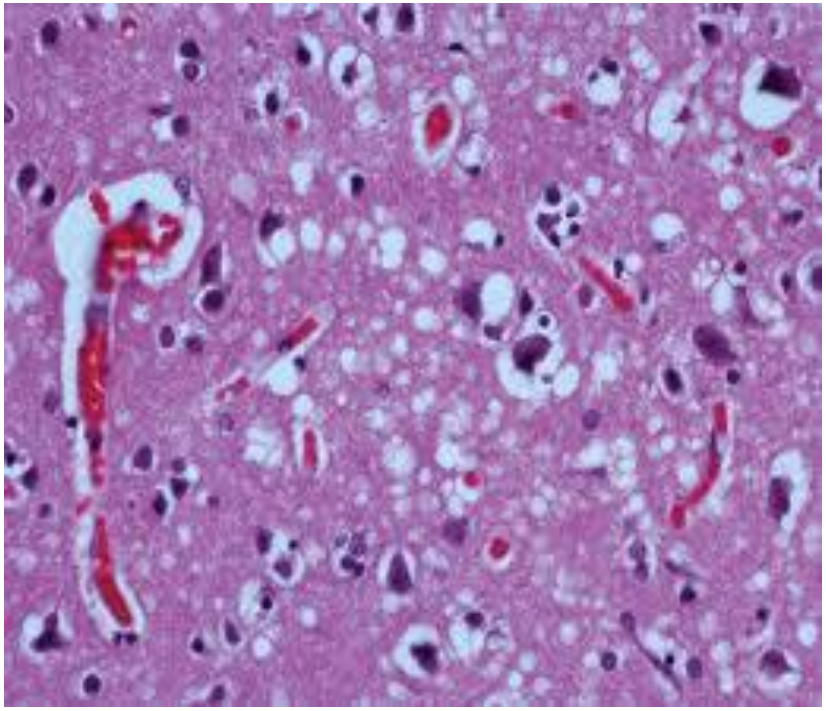
Normal Conformer

Rogue Conformer
(speculative)









Molecular genetics markers of selected diseases

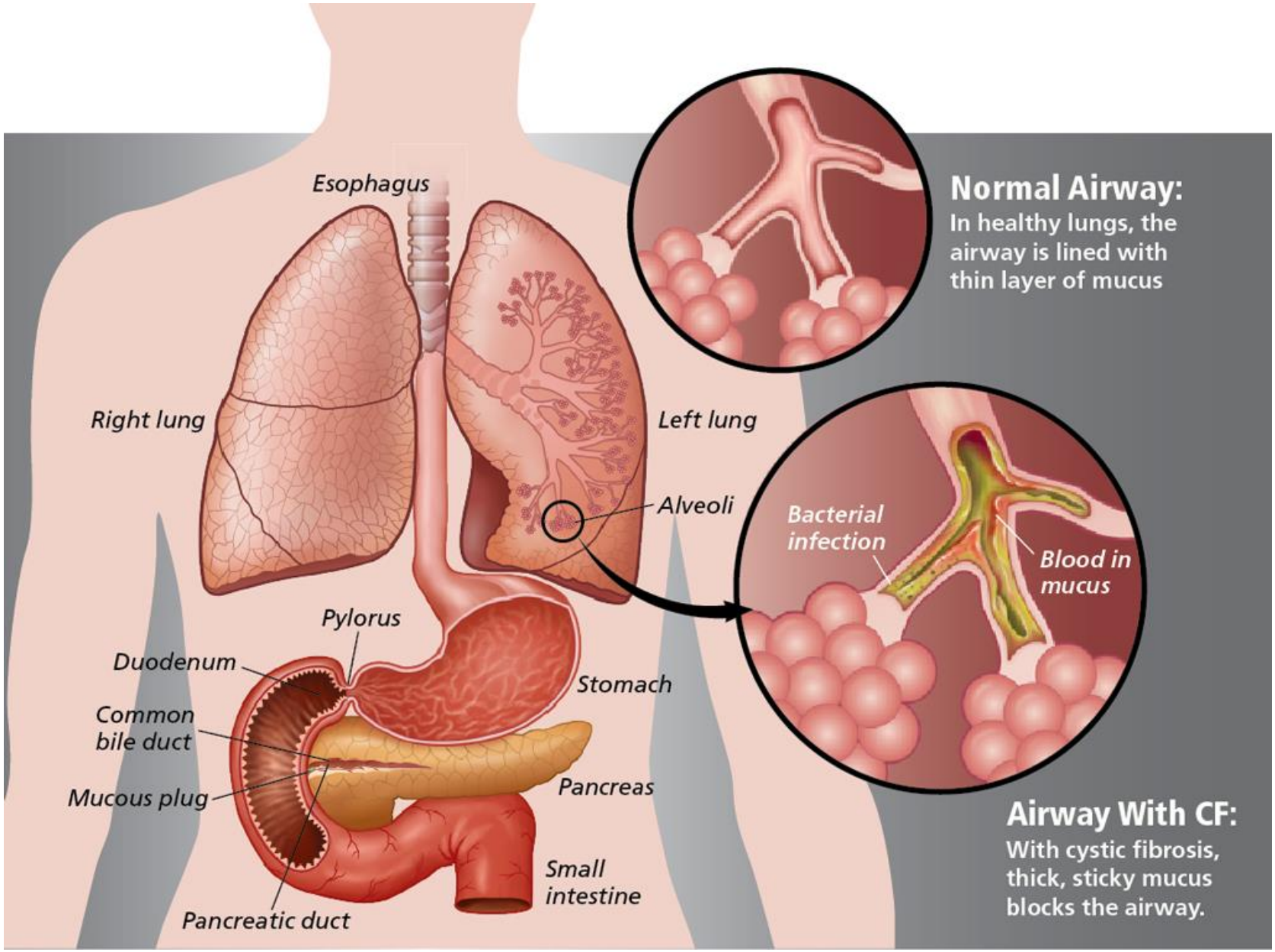
Pulmonary diseases

Cystic fibrosis

- serious incurable disease, „salty children“
- occurrence 1:3000, survival up to 30 years
- each 25th human is carrier
- multiorgan disease, lung fibrosis leads to death

Causes: - mutation in CFTR gene (27 exons)
- greatest impact on phenotype: 10, 11, 12, 20, 21, 22
- defect in Na^+ ion transfer through membrane of epithelial cells

Diagnostics: **PCR**


Esophagus
Right lung
Left lung
Alveoli
Pylorus
Duodenum
Stomach
Common bile duct
Mucous plug
Pancreas
Small intestine
Pancreatic duct
Normal Airway:
In healthy lungs, the airway is lined with thin layer of mucus
Bacterial infection
Blood in mucus
Airway With CF:
With cystic fibrosis, thick, sticky mucus blocks the airway.

Molecular genetics markers of selected diseases

Prenatal diagnostics

Invasive: (= invasion into amnion) AMC, cordocentesis, fetoscopy

Non-invasive: ultrasound, biochemical screening

biochemical screening – determination of markers level in maternal serum

Molecular genetics markers of selected diseases

Prenatal diagnostics

- performed in 1. and/or 2. trimester

First trimester

PAPP-A protein

βhCG

-sensitive to +21, +18

Second trimester

AFP α-1 fetoprotein

uE3 – non-conjugated estriol

SP1 – trophoblast specific β1-glykoprotein

- sensitive to cleft palate, intrauterine growth retardation

Paternity testing

Paternity testing

- genomic sequences of each human are unique
- DNA typing must be performed efficiently and reproducibly

- testing areas:

highly polymorphic loci – greater probability that two people don´t have the same locus

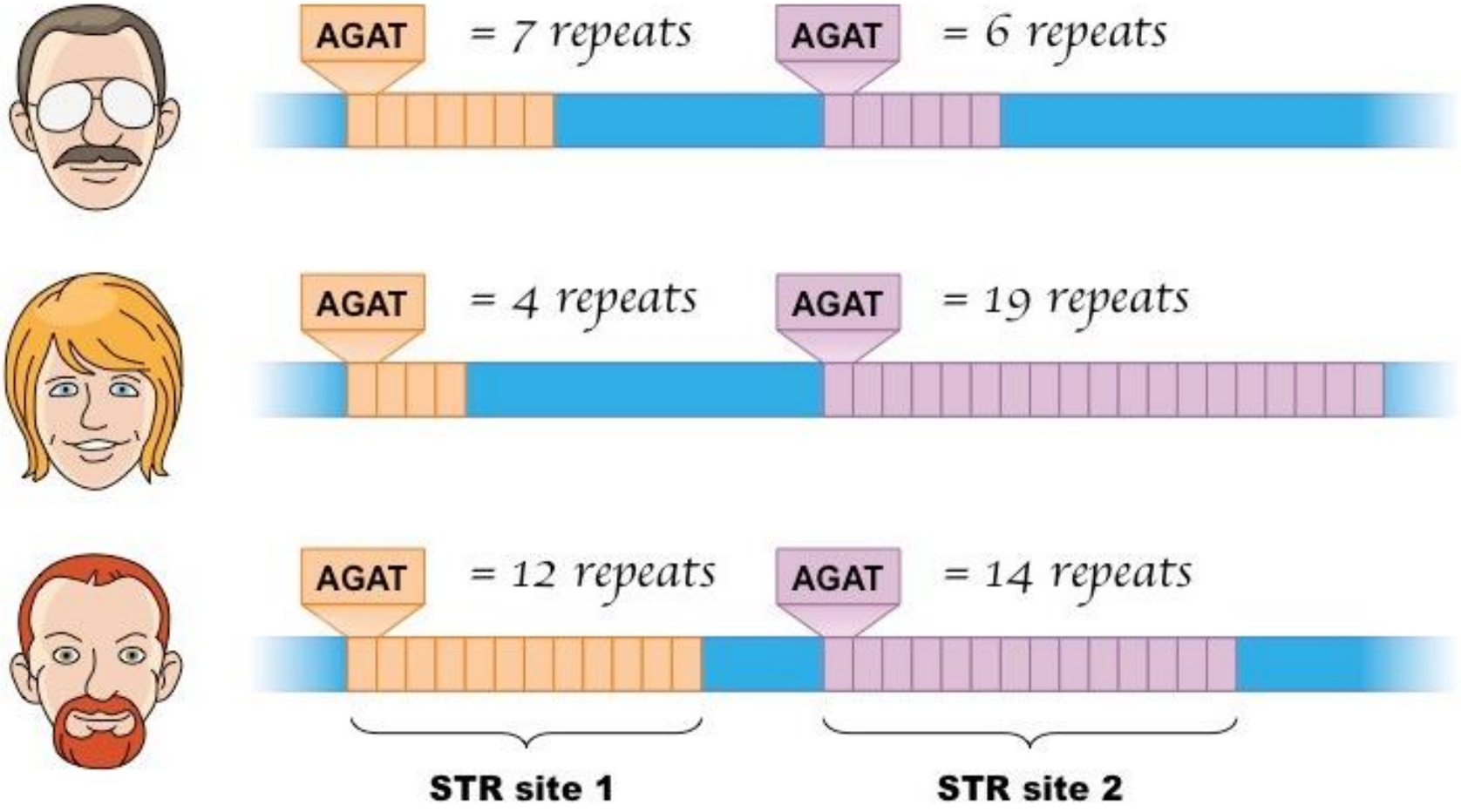
Codis loci - 13 basic loci of DNA used to create genetic profiles
- in paternity testing we performed 16 (greater certainty)
- STR markers on different chromosomes

genetic profile – combination of several highly polymorphic loci

Method: **multiple PCR**

Locus	Chromosome location	Repeat motif
D8S1179	8q	TCTA
D21S11	21q11-21	TCTA
D7S820	7q11.21-22	GATA
CSF1PO	5q33.3-34	AGAT
D3S1358	3p	TCTA
TH01	11p15.5	AATG
D13S317	13q22-31	TATC
D16S539	16q24-qter	GATA
D2S1338	2q35-37.1	TGCC
D19S433	19q12-13.1	AAGG
vWA	12p12-pter	TCTA
TPOX	2p23-pter	AATG
D18S51	18q21.3	AGAA
D5S818	5q23.3-32	AGAT
FGA	4q28	TTTC

Comparative STR Lengths at Two Specific Loci



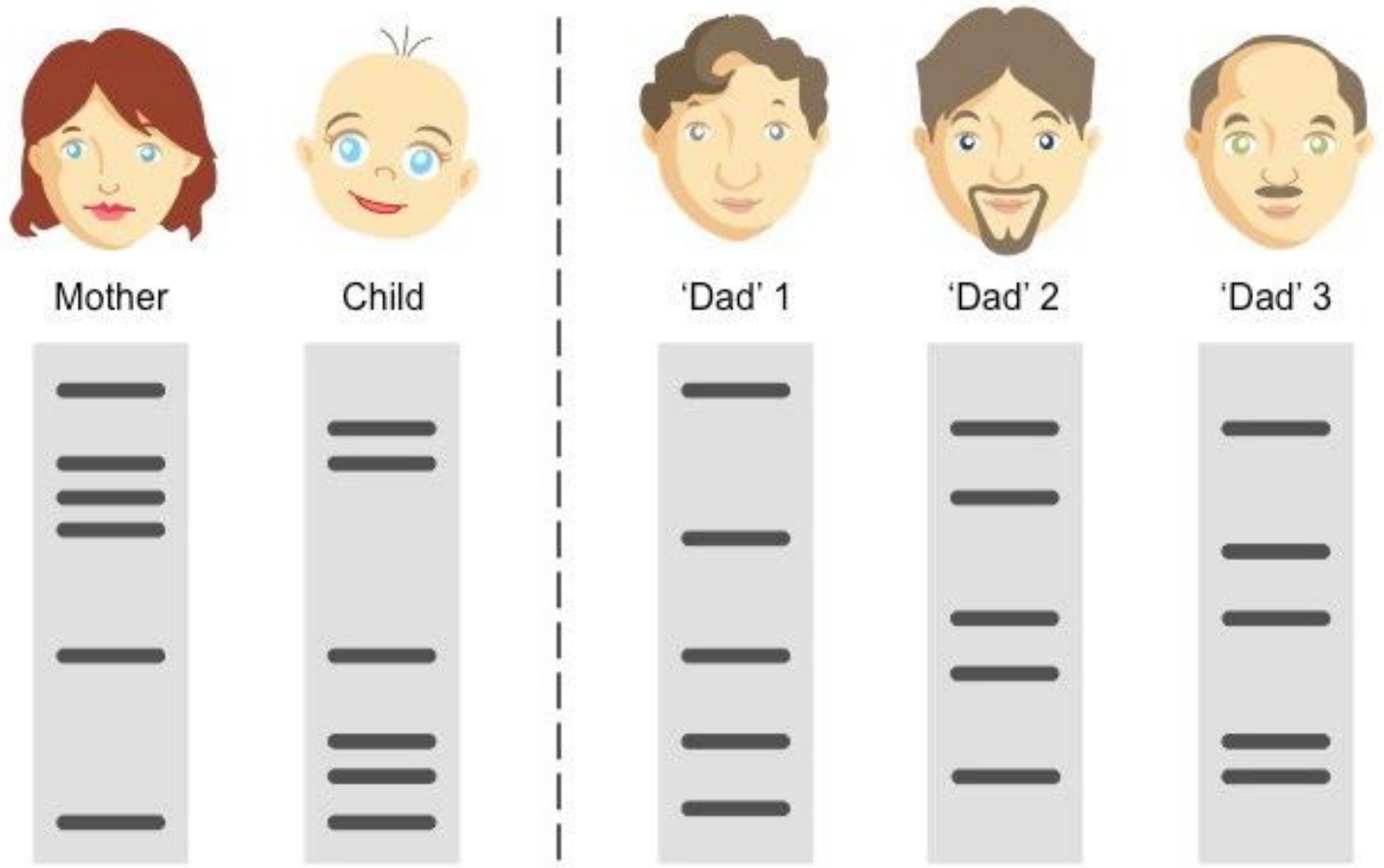
Paternity testing

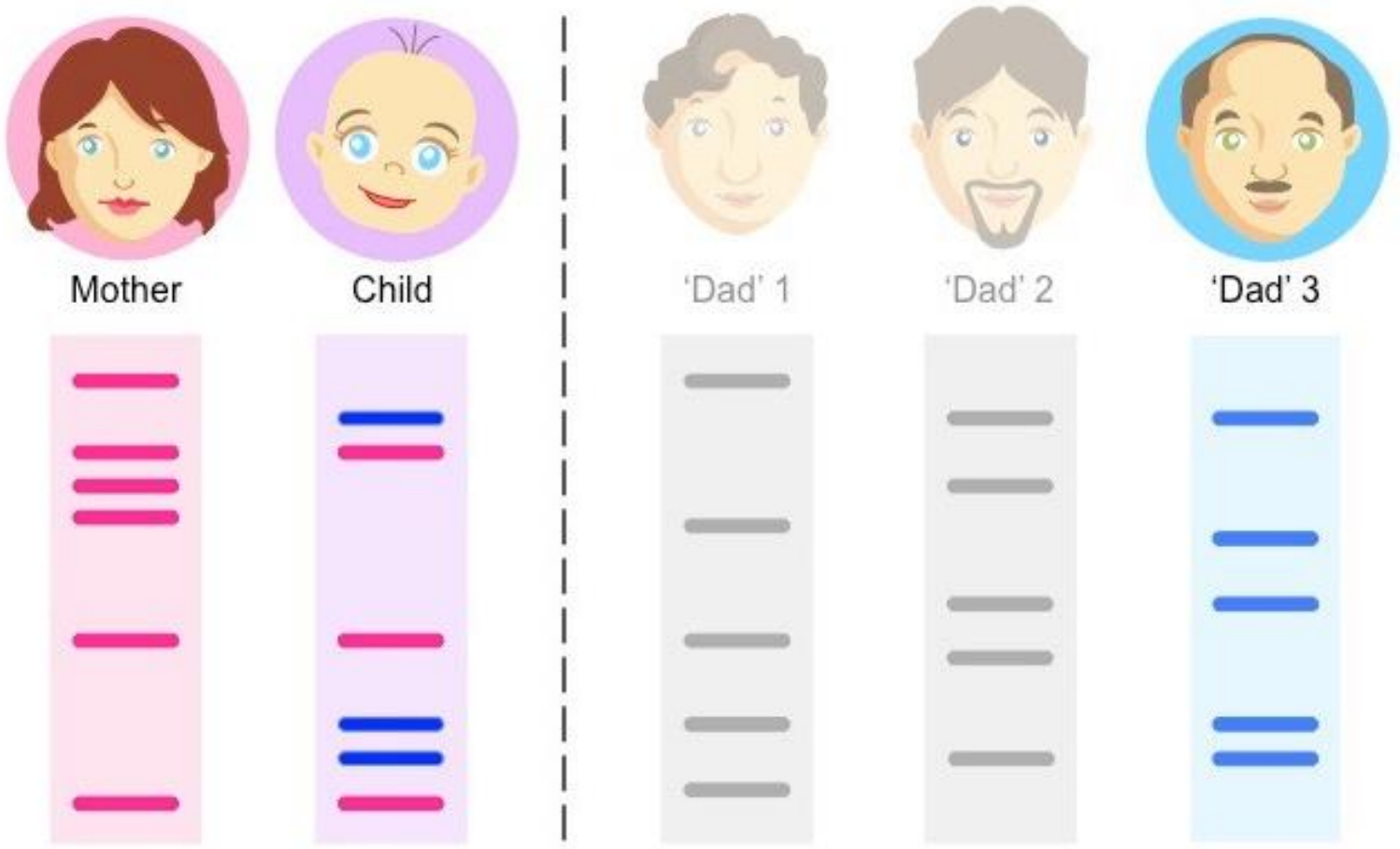
paternity index – in each population allele occurs with another frequency
- the higher the frequency, the lower the index

paternity confirmation - by multiplying paternity indices for each locus get total loci paternity index, which will be converted into the probability (99.9% accuracy)

paternity exclusion - a child has a combination of markers, that the father does not have (paternity can be 100% confidently excluded)

	Mother	Child	Potential father 1	Potential father 2
1. Locus of DNA	28 30	28 31	29 31	29 30
2. Locus of DNA	9 10	10 11	11 12	8 14
3. Locus of DNA	14 15	14 16	15 16	16 16
4. Locus of DNA	14 16	14 15	15 17	14 17


Mother
Child
'Dad' 1
'Dad' 2
'Dad' 3


Mother
Child
'Dad' 1
'Dad' 2
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Thank you for your attention!