Introduction in medical genetics 7

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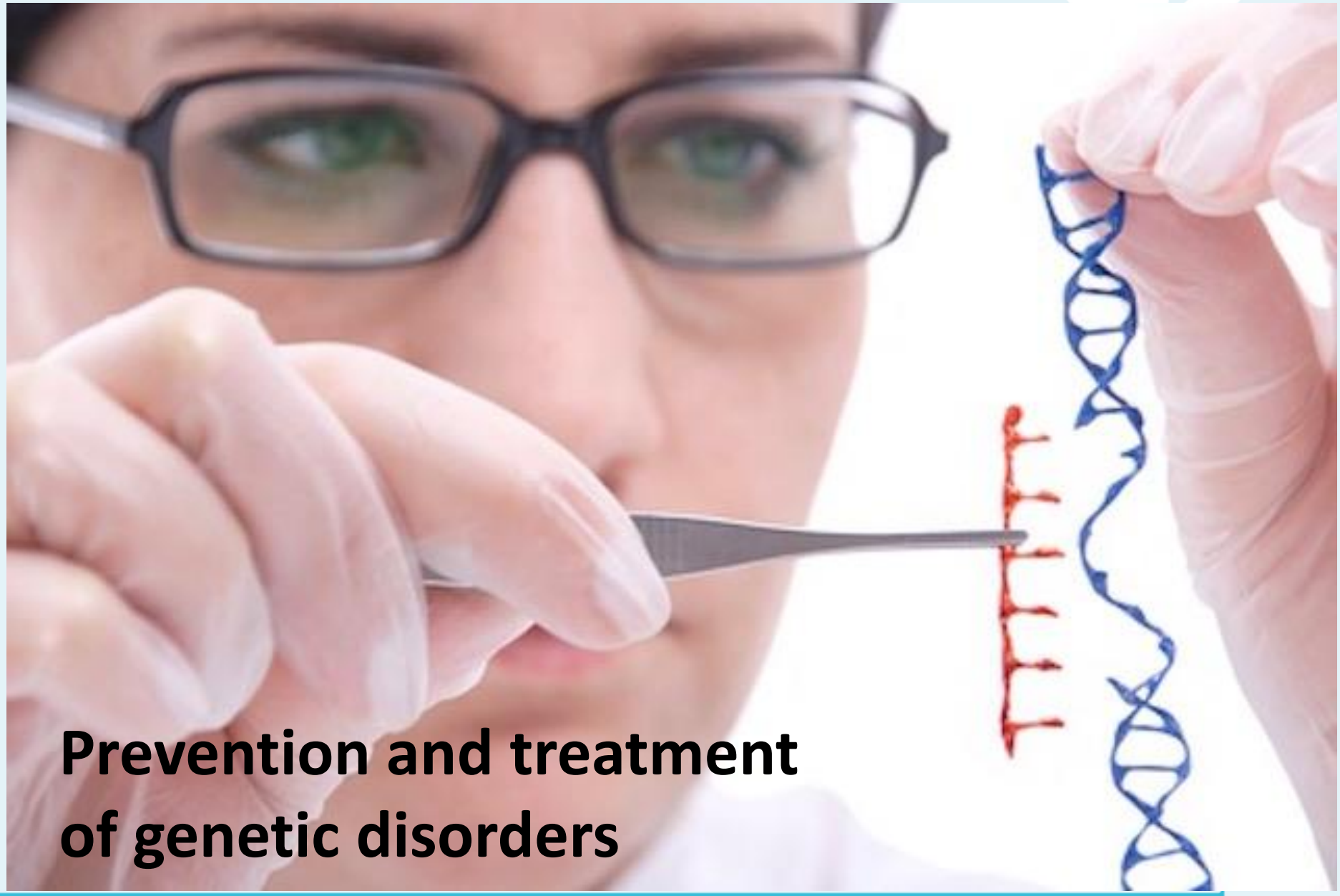
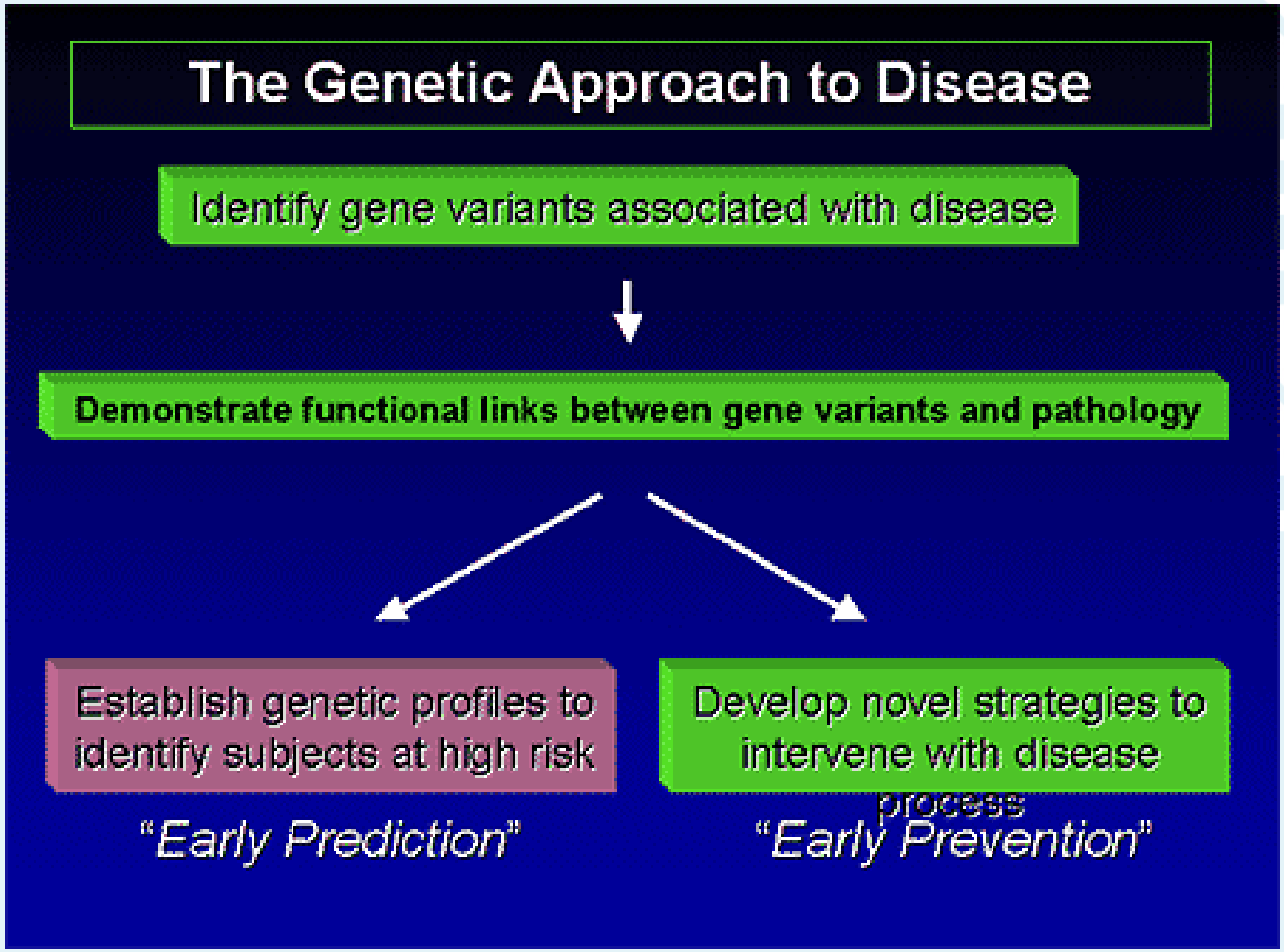
Slovenská zdravotnícka univerzita,
Bratislava, 3.4.2017

reprogen
REPRODUKČNÁ GENETIKA


Prevention and treatment
of genetic disorders


The Genetic Approach to Disease
Identify gene variants associated with disease
Demonstrate functional links between gene variants and pathology
Establish genetic profiles to identify subjects at high risk
"Early Prediction"
Develop novel strategies to intervene with disease process
"Early Prevention"

Current therapy and management of genetic disorders

Prevention

Metabolic manipulation

Gene product replacement

Cell or organ transplantation

Reconstructive surgery

Gene therapy – correction of basic genetic abnormality

Every person and every couple having children is at some risk of seeing a disorder with a genetic component suddenly appear

1. Screening of individuals and couples known **to be at significant or high risk because of positive family history** – **family (targeted) screening**
   - it includes: **carrier (heterozygote) screening**
     presymptomatic testing

2. Screening offered to a general population, who are at low risk – **community (population) screening**
   - genetic testing on an equitable basis to all relevant individuals in a defined population
   - goals: a. To enhance autonomy by enabling individuals **to be better informed about genetic risks and reproductive options**
     b. **Prevention of mobidity resulting from genetic disease** and alleviation of the suffering

- *prenatal genetic screening program, premarital screening in some populations, preimplantation genetic screening of aneuploidy in early embryos, neonatal screening of inherited disorders, single gene disorders screening*

Autosomal disorders with delayed onset or reduced penetrance – Presymptomatic diagnosis testing
Breast cancer
Familial adenomatous polyposis
Hereditary motor and sensory neuropathy type I
Hereditary non-polyposis colon cancer
Huntington disease
Inherited cardiac arrythmias
Marfan syndrome
Myotonic dystrophy
Neurofibromatosis – type I and II
Tuberous sclerosis
Von Hippel-Lindau syndrome

	Criteria for a screening program
Disease	- High incidence in target population - Serious effect on health - Treatable or preventable
Test	- Non-invasive and easily carried out - Accurate and reliable (high sensitivity and specificity) - Inexpensive
Program	- Widespread and equitable availability - Voluntary participation - Acceptable to the target population - Full information and counseling provided

	Current screening programs in Slovakia
Antenatal	- Trisomy 13, 18, 21 - Structural abnormalities *(fetal anomaly screening at 18 – 20 weeks´ gestation*
Newborn	CH - Congenital hypotyreosis CAH - Congenital adrenal hyperplasia CF - Cystic fibrosis PKU - Phenylketonuria MSUD - Leucinosis MCAD - Medium-long chains KK LCHAD – long 3-OH-acyl-CoA KK VLCAD - Very long chains KK CPT-I; CPT-II: Carnitine-palmitoyl-CoA transferase Glutaric aciduria type I Isovaleric aciduria
Adult	- Breast cancer - Cervical cancer - Colon cancer
Donors of oocytes, sperm and embryos	- Karyotype - Cystic fibrosis

Potential advantages and disadvantages of genetic screening

Advantages
- Informed choice
- Improved understaining
- Early treatment when available
- Reduction in births of affected homozygotes
Disadvantages and hazards
- Pressure to participate causing mistrust and suspicion
- Stigmatization of carriers (social, insurance and employment)
- Inappropriate anxiety in carriers
- Innappropriate reassurance if test is not 100% sensitive

	Prevention of genetic disorders
Primary prevention	**Preconception single gene disorders screening**
	Premarital genetic screening – some populations *(Ashenazi Jewish, Sicilia)*
	Preimplantation genetic diagnosis *(single gene disorders and translocations)*
	Preimplantation genetic screening of aneuploidy in early embryos: - *improve success of low-risk infertile couples by IVF treatment* - *prevention of chromosomal trisomy in baby*
Secondary prevention	**Prenatal diagnostics**

Single gene disorders

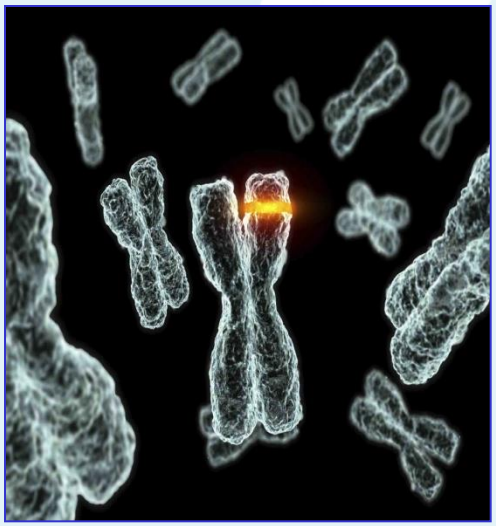
- Fenotyp effects are caused by the mutation in one gene
- **Mendelian type of inheritance**

- **7 000 single gene disorders**

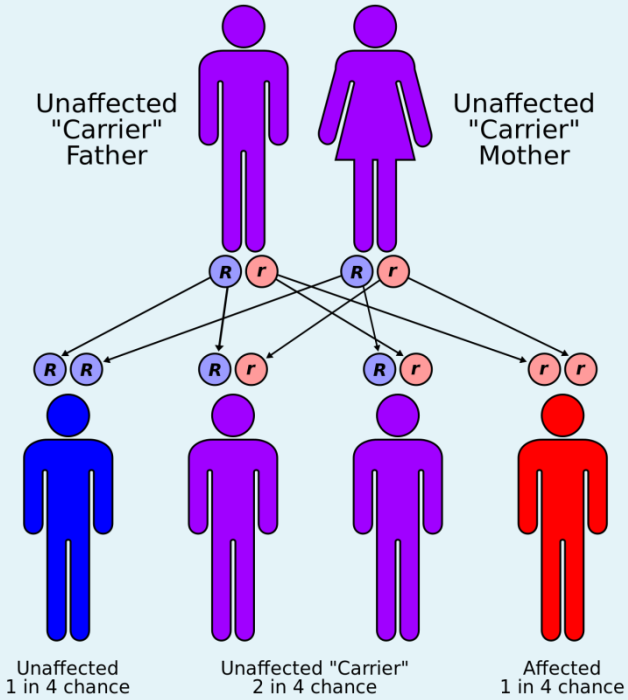
WHO: Prevalence **10 : 1000**

20% of cases of **child mortality**
in developed countries

40% of medical interventions
in children hospitals
(Kanada – Scriver, 1995)



Carrier screening of single gene diseases



single gene disorders

AR a X-linked inheritance

General population – affected **1 : 100**

Carriers

Cystic fibrosis	**1**	**: 25**	**4 %**
Spinal muscular atrophy	**1**	**: 50**	**2 %**
Alpha-/Beta-hemoglobinopathy	**1**	**: 48**	**2 %**

Carrier screening of single gene diseases

- analysis of 549 genes, 600 single gene diseases using NGS
- high risk of transmission of disease with recessive inheritance (AD or X-linked) to the next generation

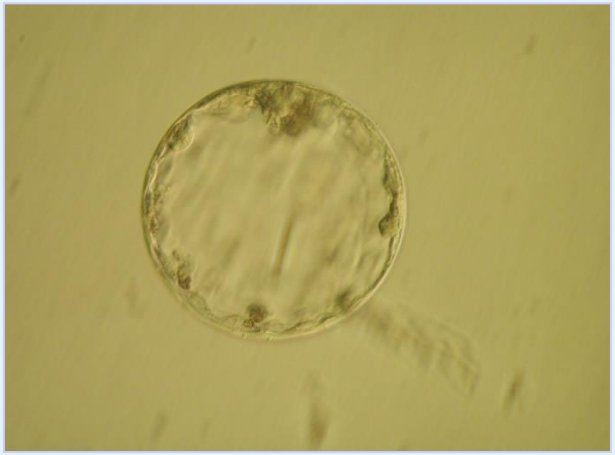
2.1 pathogenic mutation/person

7.1 % of tested persons don´t have pathogenic mutation

8% couples carry the mutation in the same gene

(so called „genetic incompatibility“)

Carrier screening of single gene diseases



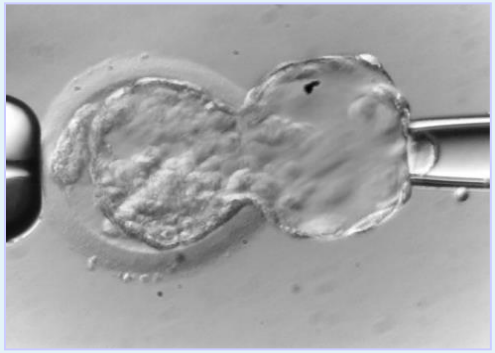
For whom?

Before natural conception

Before assisted conception

Oocytebanking and spermbanking

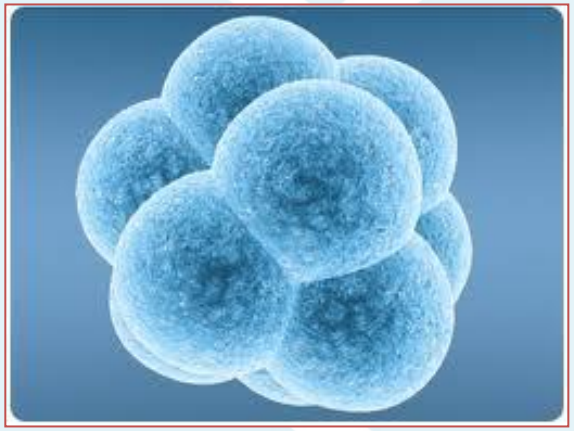
Preimplantation genetic diagnostics



1 : 100

1 : 100 000

PGD / PGS



Preimplantation genetic diagnostics

- analysis of genetic disorders in early human embryos before their embryotransfer in uterus as a prevention of single gene disorders

- Introduced in routine: ***1990 – adrenoleukodystrophy***

Preimplantation genetic diagnostics – prevention of genetic disorder transmission to the next generation

- single gene disorders
- chromosomal translocations

Preimplantation genetic screening af aneploidy – prevention of transfer of chromosomally abnormal embryos of „poor responder“ couples

- older women (35+)
- repeated unsuccessful implantation of embryos
- repeated spontaneous miscarrages
- male infertility

PGD medical indications

Preimplantation genetic diagnosis

- Diagnostics of single gene disorders
- Diagnostics of translocations
- Diagnostics of late-onset genetic diseases or cancer diseases occuring in adulthood
- HLA typization of embryos
- Mitochondrial diseases

PGD - steps

Haplotype analysis of family

– PGD set-up

IVF/ICSI

Hormonal stimulation

Oocyte pick-up

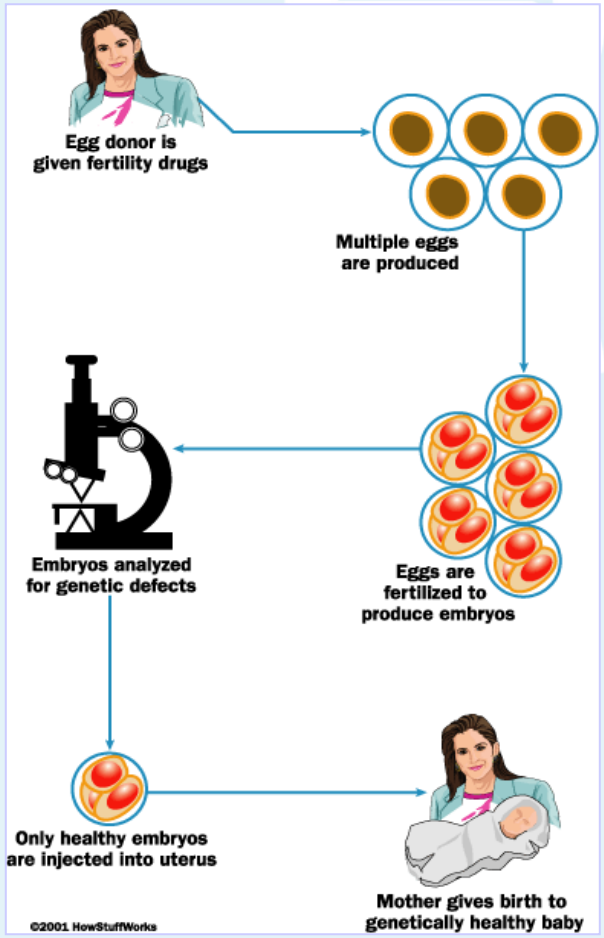
Fertilization

Embryo biopsy – Day 3,

Day 5 or blastocentesis

Genetic analysis

IVF – Embryo transfer



PGD/PGS schematic representation

Day 0

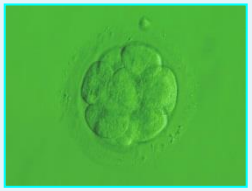
Oocyte pick-up

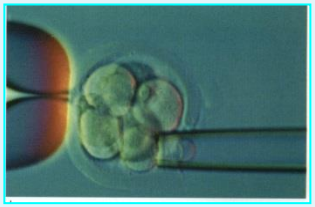
Day 1

Fertilisation

Day 3

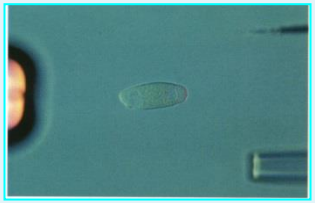
Biopsy of blastomere

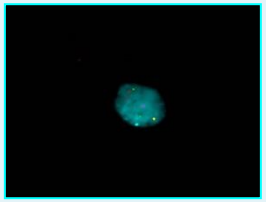




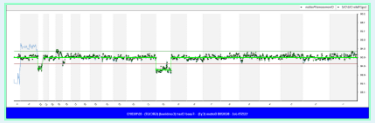
Day 3 + Day 4

FISH, aCGH,
PCR-PGD





results, report



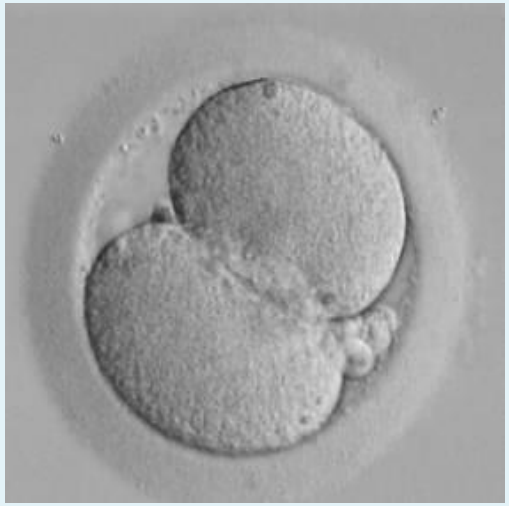
Day 5

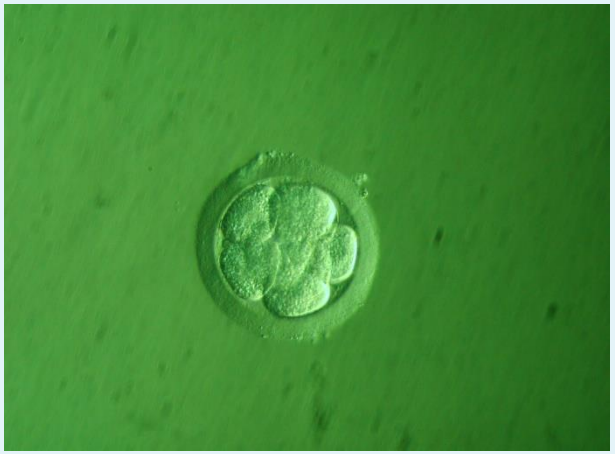
Embryo transfer

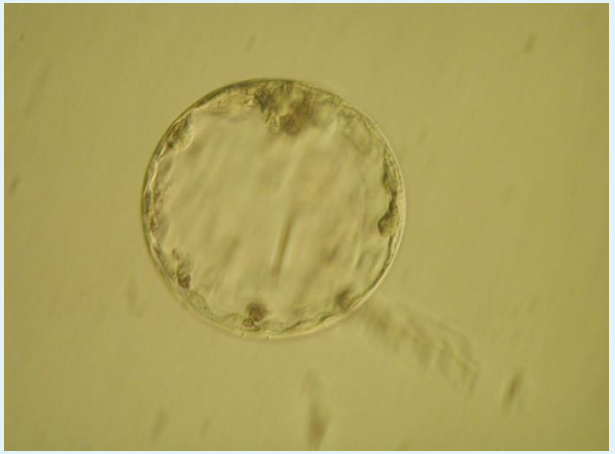
Biopsy of
trophoectoderm

vitrification of biopted embryos
aCGH, NGS
ET in next menstrual cycle

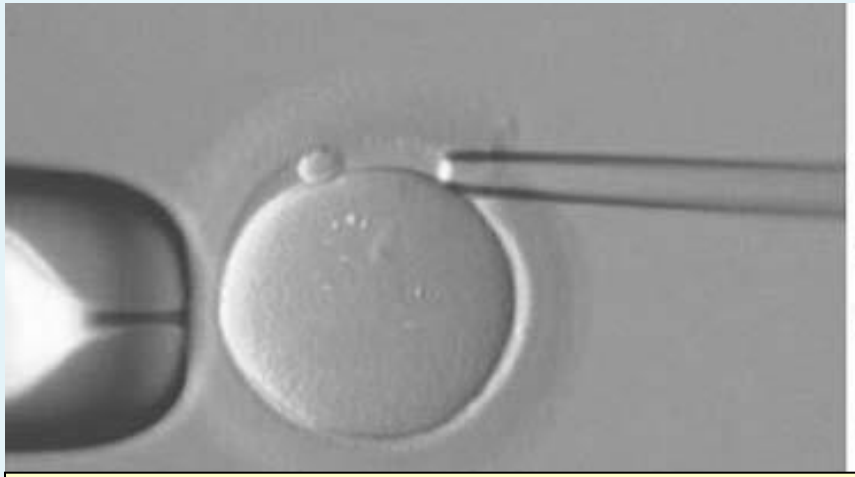
Embryo culture

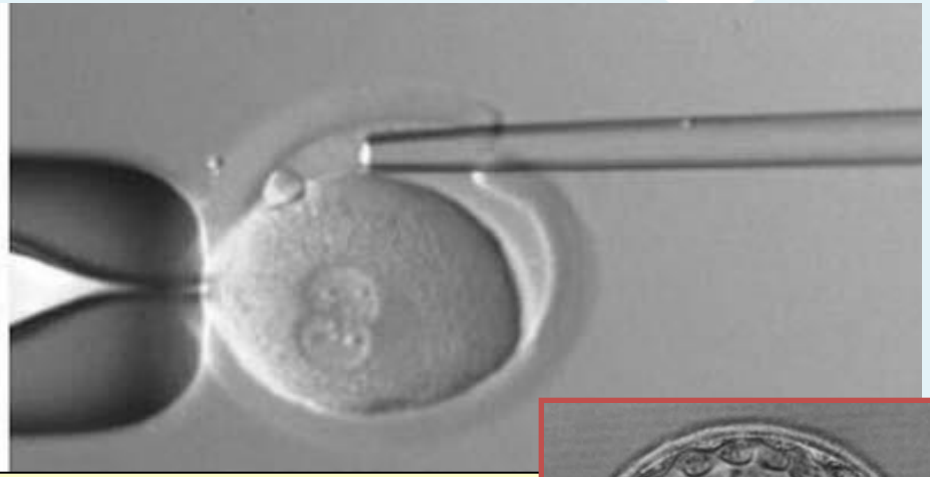






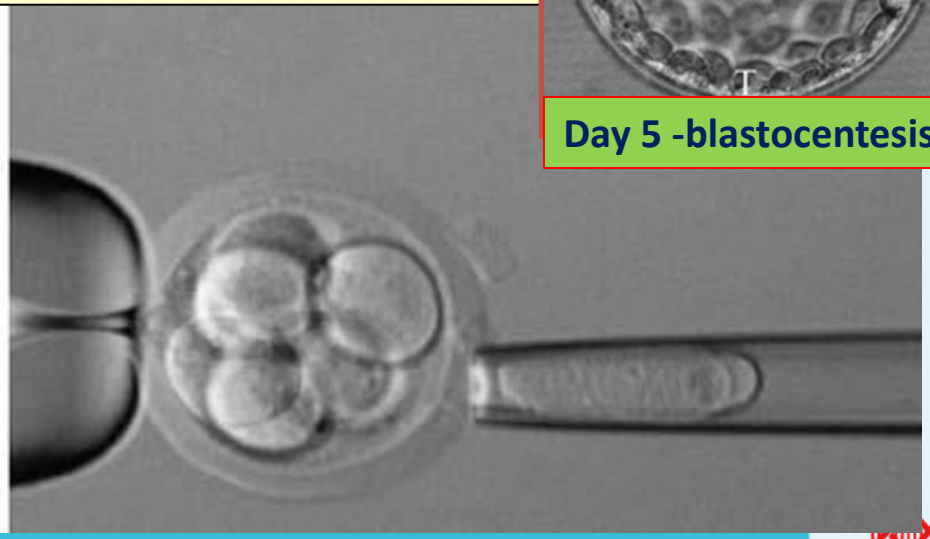
Embryo biopsy





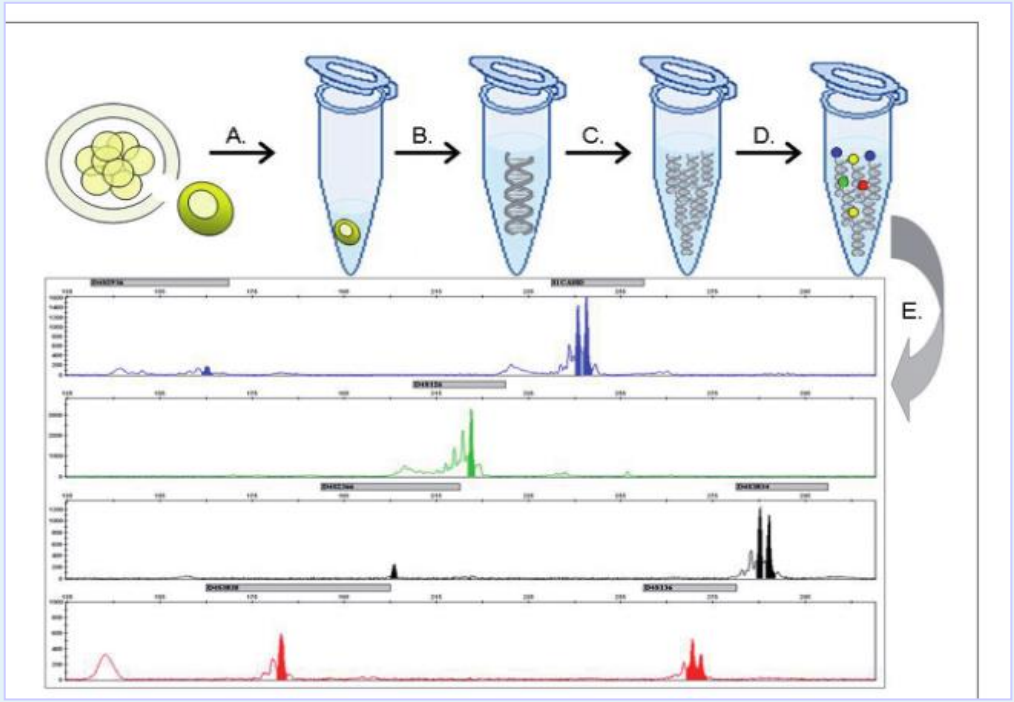
1st polar body **2nd polar body**

Trofoectoderm of blastocyst – Day 5 **Blastomere - Day 3**



Day 5 -blastocentesis

PCR – PGD steps



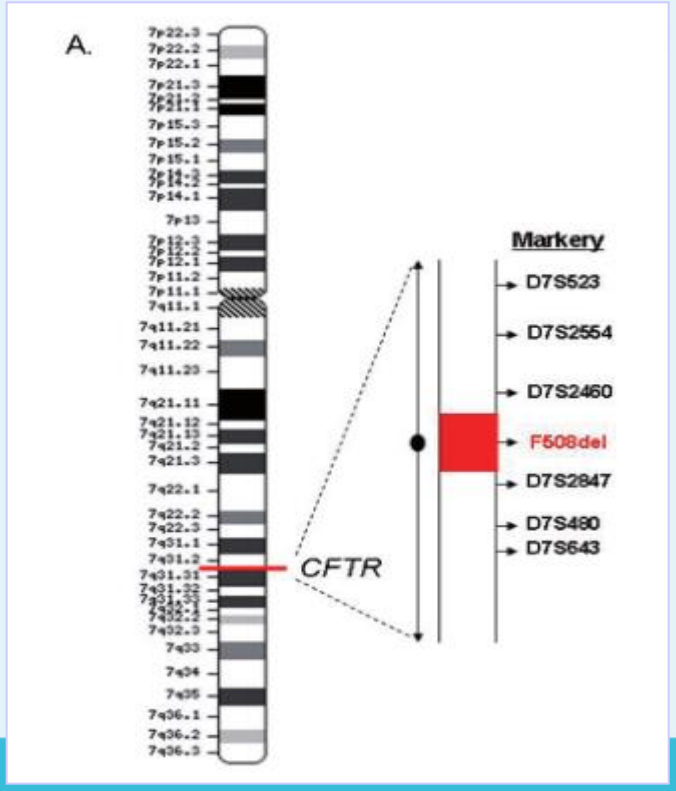
- **Bioptovaná bunka (bunky)**

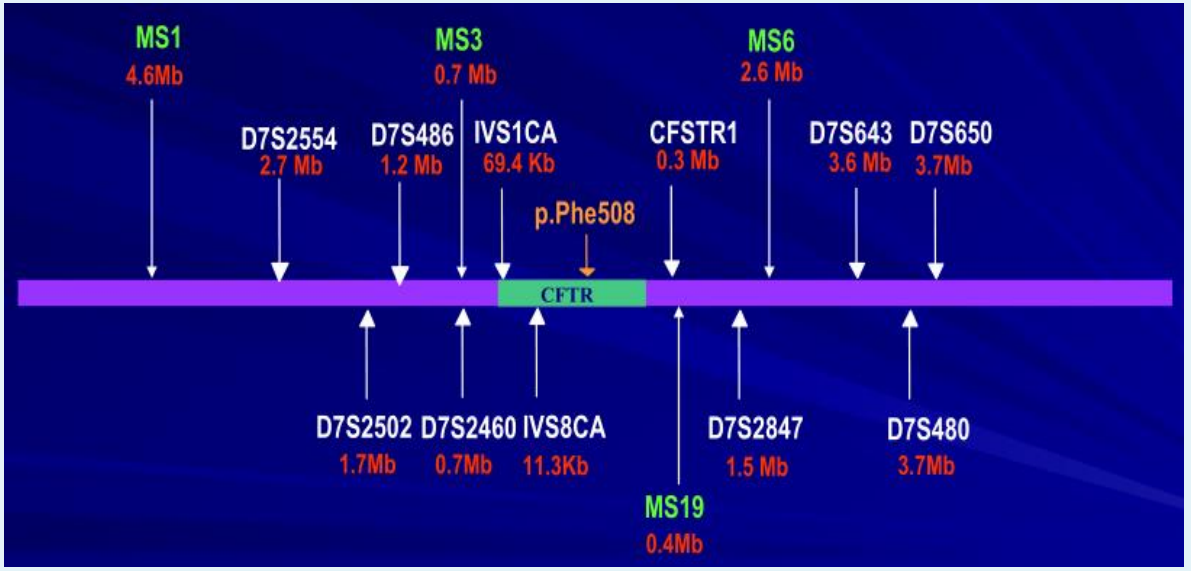
  z embrya je prenesená do lyzačného roztoku.
- **Genóm jedinej bunky je uvoľnený z jadra**
- **Genóm je mnohonásobne namnožený procesom celogenomovej amplifikácie (WGA)**
- **WGA produkty sú amplifikované multiplexnou fluorescenčnou PCR reakciou (detekujú sa sekvencie špecifických markerov, ktoré sú vo väzbe s vyšetrovaným génom)**
- **Fragmentačnou analýzou pomocou kapilárnej elektroforézy je určený genotyp vyšetrovaného embrya**

Haplotype analysis

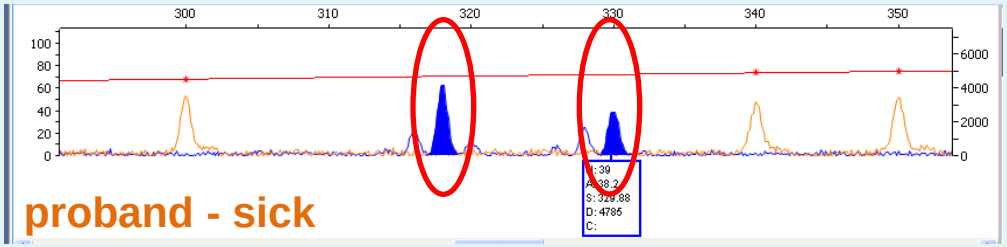
Allelic drop-out risk

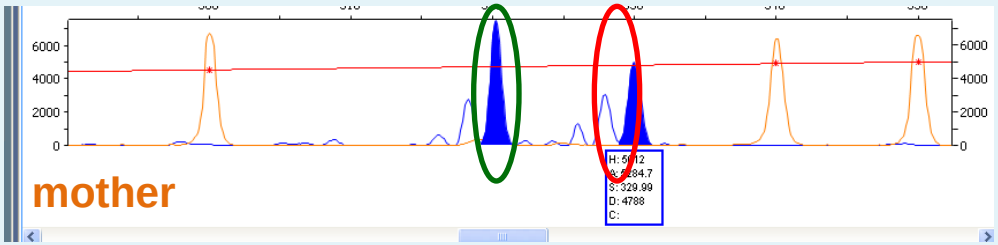
Analysis of 5 fully informative DNA markers linked to the mutation

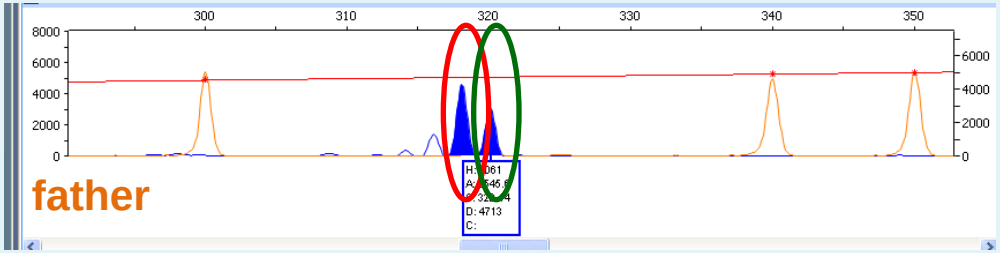


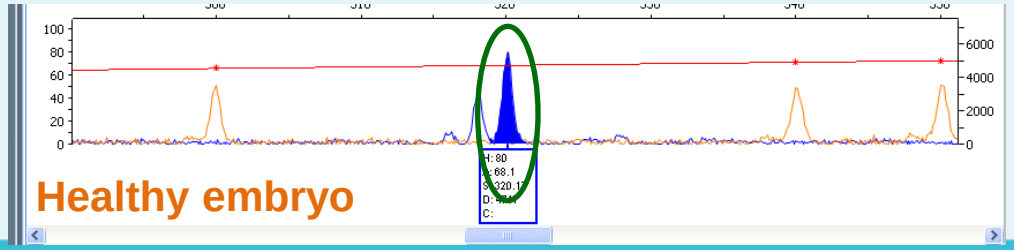


Haplotype analysis of the family







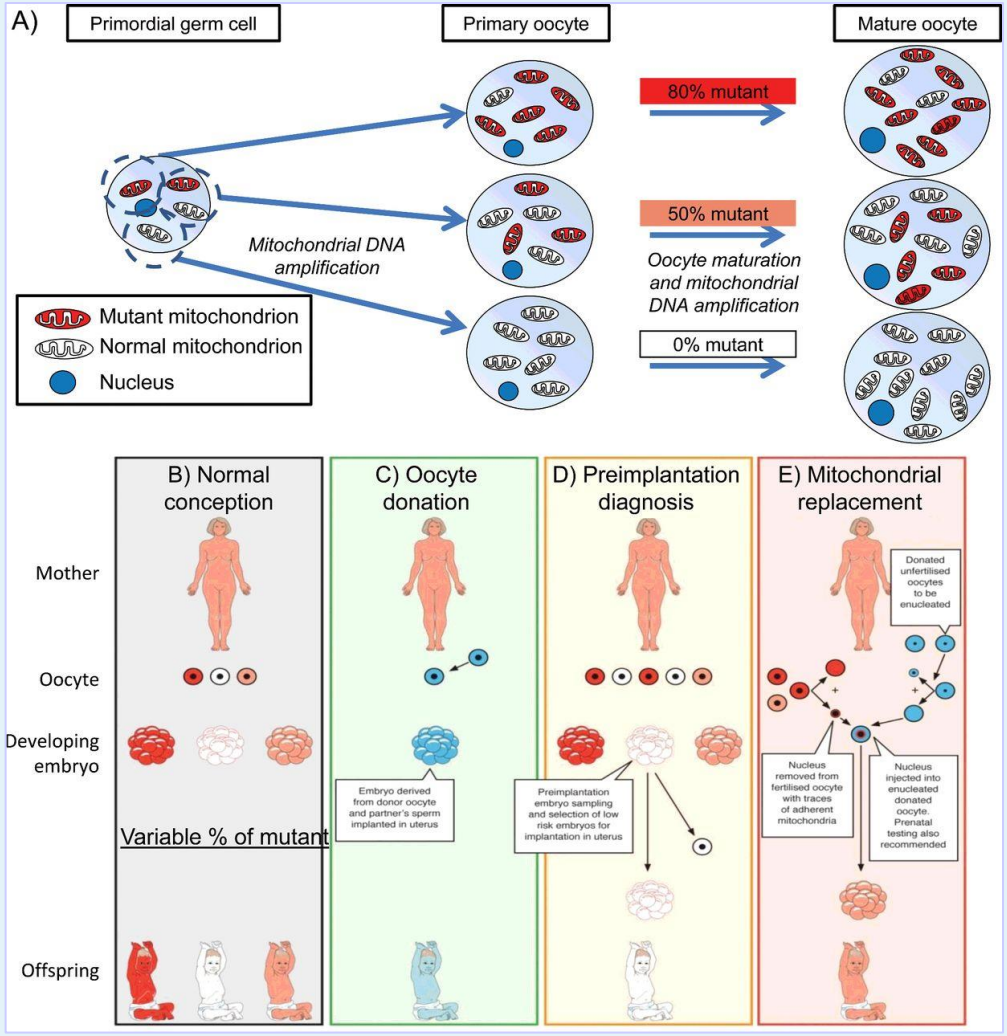


Marker –IVS10CA

IVS10CA	Alela 1	Alela 2
Proband - affected	318	330
Mother	320	330
Father	318	320
Healthy embryo	320	320

Analysed 12 DNA markers before, inside and after CFTR gene

Transmission of mtDNA disorders and the strategies of prevention



Alan Diot et al. Biochm. Soc. Trans. 2016;44:1091-1100

PGS medical indications

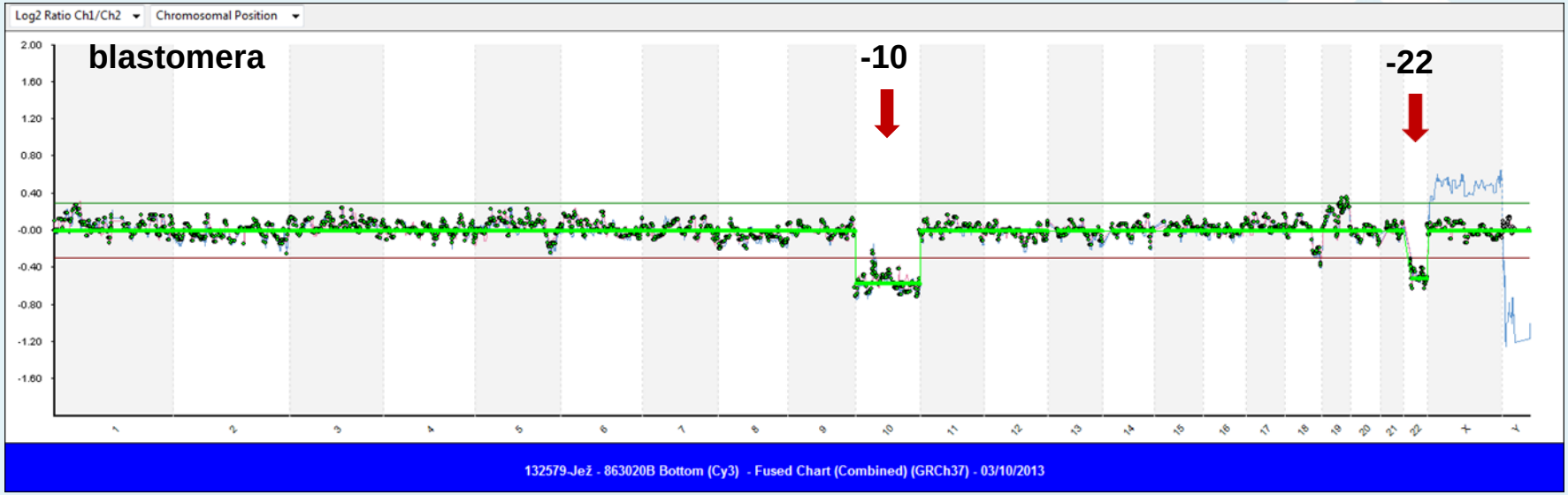
Preimplantation genetic screening of aneuploidy

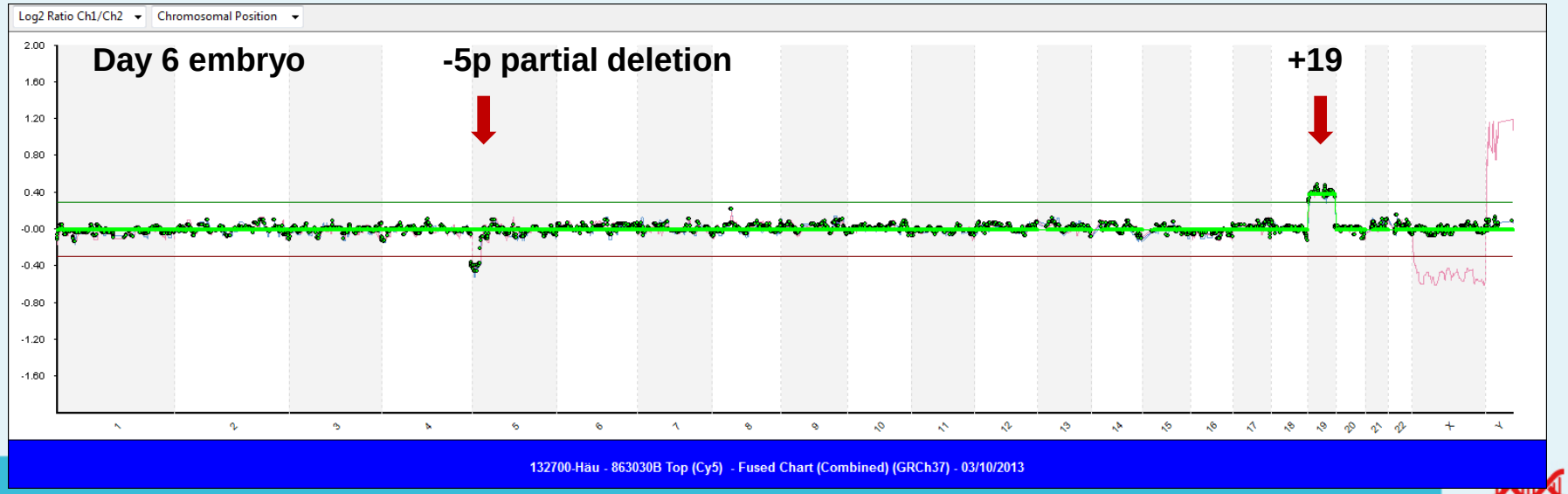
FISH: 8 chromosomes

aCGH / NGS: all chromosomes

- Women older than 35 - 37 years
- Repeated unsuccessfull IVF cycles
- Repeated spontaneous miscarriages
- Trisomic fetus in previous pregnancy (Down syndrome)
- Male infertility (OAT gravis, TESE/MESA)

Examples of aCGH results





Spontaneous miscarriages I.
Aneuploidy

Natural conception: 15 %

ART: 23 – 37 %

PGS: 12 - 15 %

2 x ⬇ comparing to ICSI

Spontaneous miscarriages II. Translocations

Spontaneous miscarriages:

Natural conception	87 %
PGD	18 %

5 x ↓

Take-home-baby rate:

Natural conception:	11,5 %
PGD:	81,4 %

7 x ↑

Trisomy child delivery

Natural conception:

2,6 % **trisomy 13, 18 a 21**

(chorionic villi examinations)

PGD cycle:

0,6 % **trisomy 13, 18 a 21**

independent on type of hormonal stimulation

4 x ↓

Importance of PGD / PGS

Diagnosis	**- diagnosis of chromosomal anomaly/single gene disease according to the patient´s medical indication**
Prevention	**- decreasing risk of spontaneous miscarriage in IVF couples to 15%, increase pregnancy rate with healthy embryo**

PREVENTION OF DELIVERY OF CHILD WITH GENETIC DISORDER !

Treatment	**- higher effectivity of infertility treatment in poor prognosis patients**
Prognosis	**- prognosis & change of infertility treatment in next IVF cycle**
Finance	**- next examinations & IVF treatment**
Psychology	**- psychic status of infertile couple**

Premarital screening

- Ashkenazi Jewish carrier testing

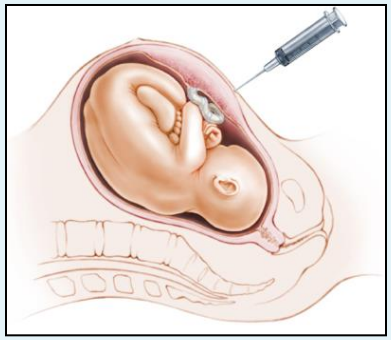
Table 1
Diseases included in Ashkenazi Jewish carrier testing

Disease	Carrier frequency in Ashkenazi Jews	Clinical features	Life expectancy
Bloom Syndrome (BS)	1/110	Dysmorphic features Reduced fertility Predisposition to malignancy (leukemia)	Childhood to young adulthood
Canavan disease (CD)	1/59	Progressive neurodegeneration	Childhood to young adulthood
Cystic fibrosis (CF)	1/33	Reduced fertility Pulmonary disease Pancreatic insufficiency	Childhood to young adulthood
Familial dysautonomia (FD)	1/32	Progressive neurodegeneration Autonomic dysfunction	Childhood to young adulthood
Fanconi anemia (FA)	1/89	Dysmorphic features Pancytopenia Predisposition to malignancy (leukemia)	Childhood to young adulthood
Gaucher disease type 1 (GD)	1/13	Thrombocytopenia, anemia and bone lesions	Normal
Hearing loss (DFNB1)	1/21	Hearing loss	Normal
Mucolipidosis type IV (MLP)	1/127[27]	Dysmorphic features Progressive neurodegeneration	Childhood to young adulthood
Niemann-Pick type A (NP)	1/90[28]	Progressive neurodegeneration	Early childhood
Tay-Sachs disease (TS)	1/31	Progressive neurodegeneration	Early childhood

All data from Online Mendelian Inheritance in Man, except when noted.

USG / age / biochemical screening

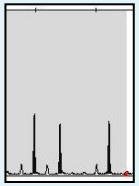
Invasive prenatal genetic test



amniocentesis / CVS

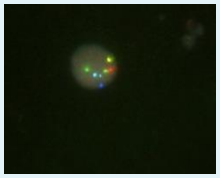
RAPID PND

QF-PCR

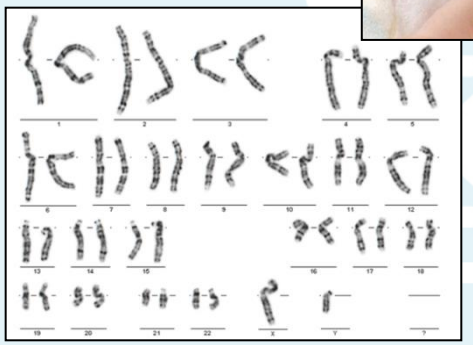


or

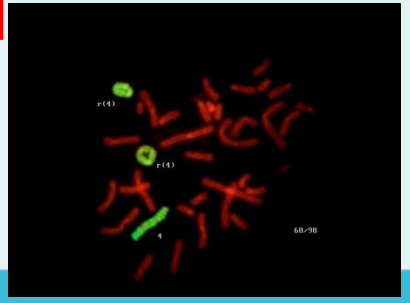
FISH



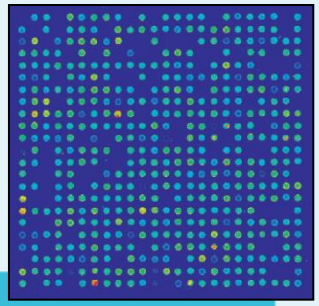
KARYOTYPE



FISH

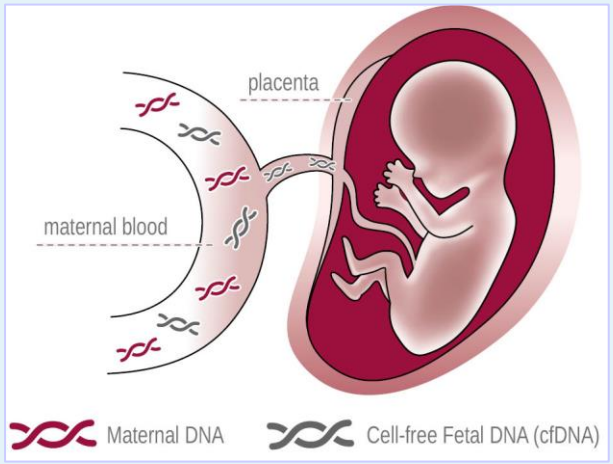


DNA chip



Risk of miscarriage: 0.5 – 1%
dg impossible before 10th week of gestation

Non-invasive prenatal genetic test



cffDNA – cell free fetal DNA

Presence of fragments of **cell-free fetal DNA (cffDNA)** in peripheral blood of pregnant woman gives us the opportunity for non-invasive prenatal testing and diagnostics

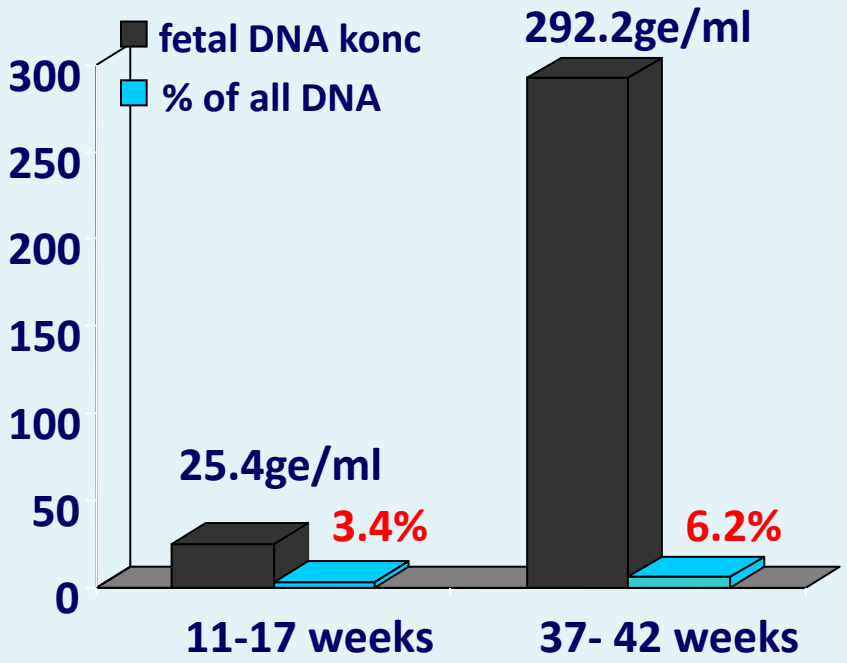
cffDNA originates from trophoblast

Testing from 10th week of pregnancy

Cell-free DNA extracted from blood of pregnant woman is a mix of DNA of two individuals – mother and her child and for this reason:

We can examine just these parameters, which doesn´t occur in mother

cffDNA – cell free fetal DNA



cffDNA constitutes 3-6% of all cell-free DNA (cfDNA) in the blood of pregnant woman

Minimal amount needed for analysis – 2%

cffDNA is destroyed after 16 minutes

We must take into account multiple fetus and vanishing baby

Sampling and cell-free fetal (cffDNA) extraction from maternal blood

2 - 5ml of blood in EDTA,
Centrifugation up to 24-48 hours
Or
Sampling in Streck test tubes

cffDNA extraction from maternal plasma

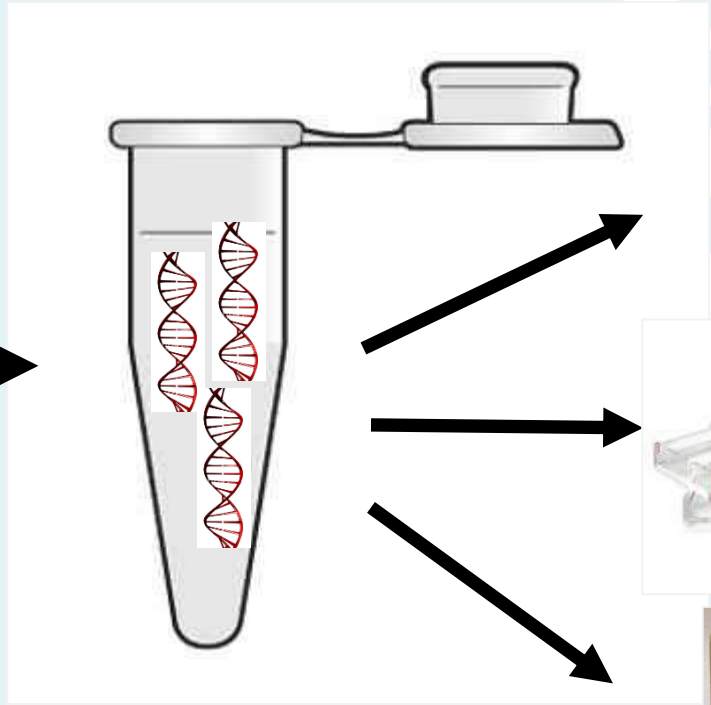
QIAGEN – Virus DSP kit

Fetal DNA

Maternal DNA

Molecular-genetic analysis

PCR



Fetal DNA

Maternal DNA

Aplications

PrenatalSafe® KARYO

PrenatalSafe KARYO +

Chromosomal abnormalities in fetus

Rhesus factor of fetus

Examination of sex

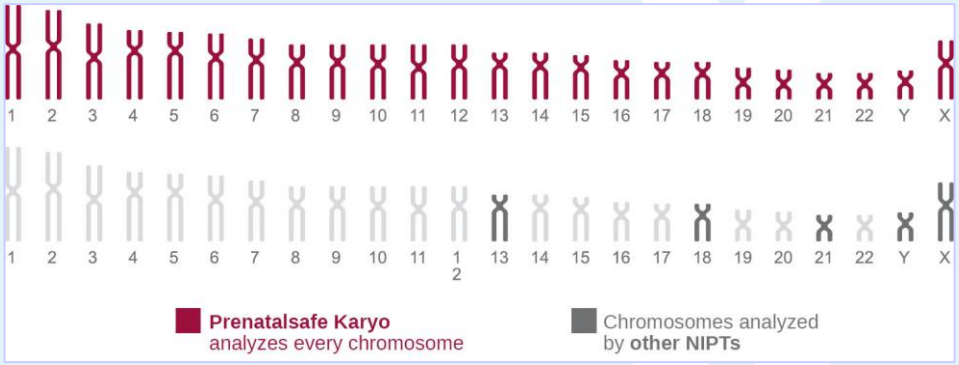
Paternity test

Non-invasive prenatal diagnostics of single gene disorders

(mutation *de novo* a transmitted from father)

PrenatalSafe® KARYO

PrenatalSafe KARYO Plus



From 10th weeks of pregnancy

Numerical abnormalities of chromosomes – aneuploidy 1 – 22, X, Y

Structural deletion or duplications – resolution 7 Mb (one G-band in karyotype)

Sex chromosome examination

Microdeletion syndromes:

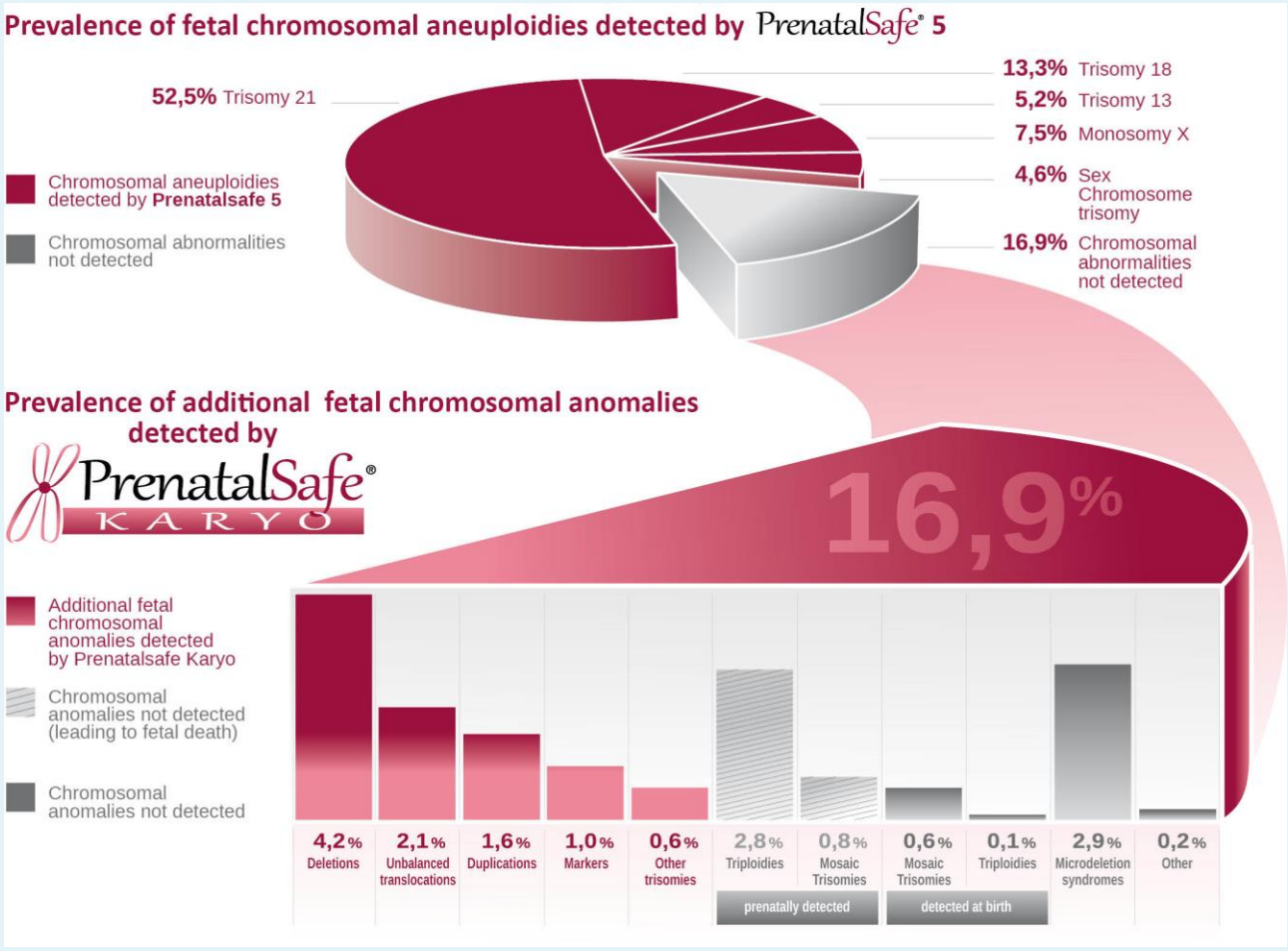
Prader Willi/Angelman syndrome – deletion 15q11.2
Di George syndrome – deletion 22q11.2
Wolf Hirschhorn syndrome – deletion 4p
Cri-du Chat syndrome – deletion 5p
deletion 1p36

It recognizes 92,6% of chromosomal abnormalities detected in prenatal development of fetus

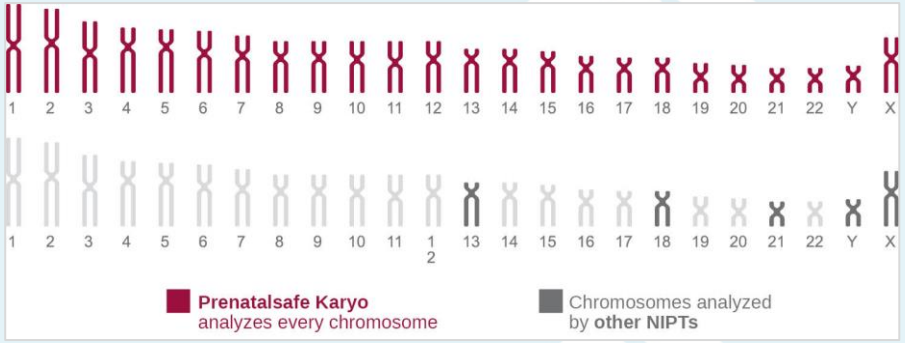
	Classical karyotyping	PrenatalSafe® KARYO
Analysis of each of chromosomes	✓	✓
Neinvasive procedure	✗	✓
Unbalanced translocations	✓	✓
Aneuploidy	✓	✓
Mosaics	✓	✗
Marker chromosomes	✓	✓
Microdeletion syndromes	✗	✗
Triploidy	✓	✗
Diagnostic test	✓	✗

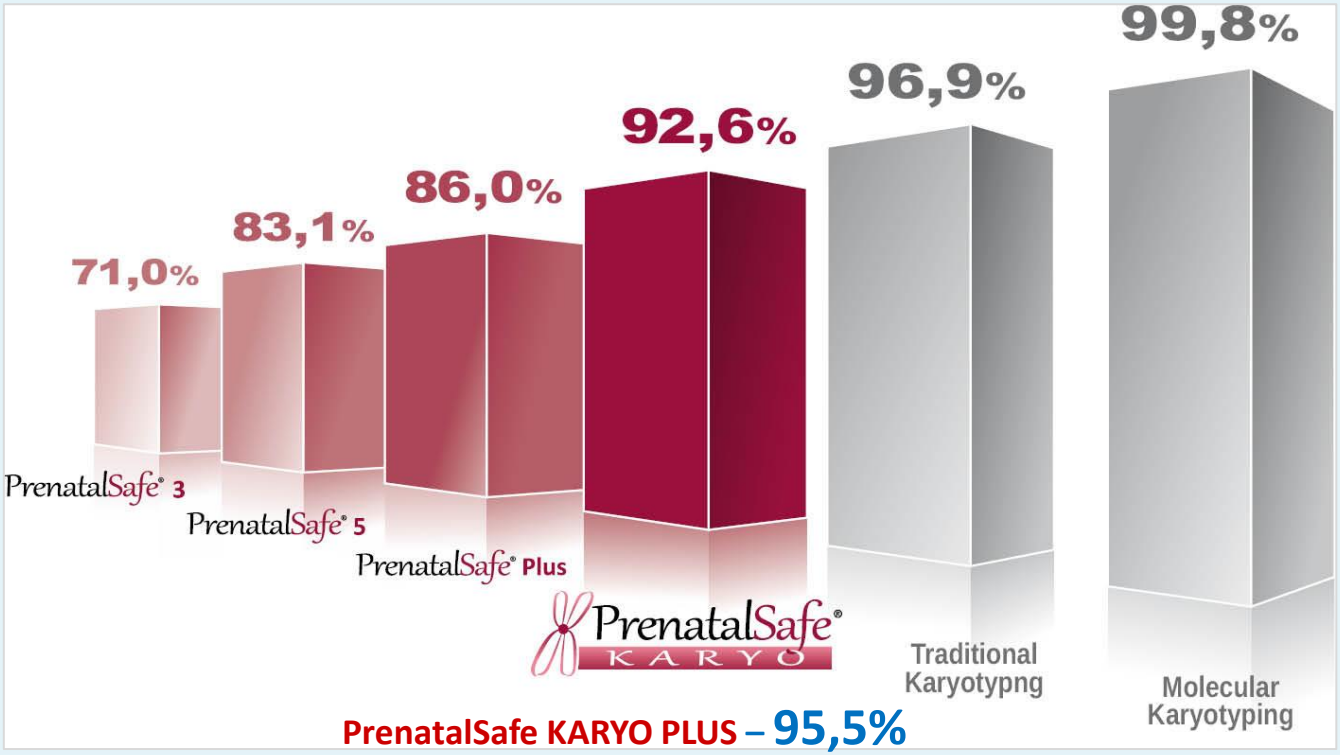
PrenatalSafe® KARYO

It detects 96,2% of chromosomal anomalies occuring at the delivery time of baby



Comparision of non-invasive prenatal tests





PrenatalSafe KARYO PLUS – 95,5%

PrenatalSafe® KARYO

Medical indications - for whom?

PrenatalSafe KARYO +

- For women with USG finding where chromosomal abnormality is suspect (numerical, structural, microdeletion syndrome)
- Occurence of balanced translocation carried by one of parents
- For women older than 35 years with negative results of biochemical screening
- For women with positive biochemical screening
- For women with chromosomal abnormality in previous pregnancy
- For women after IVF treatment including the twins
- For women with repeated spontanneous miscarriages
- For women having a risk of complications after amnioicentesis
- Psychological reason

Recommendations

Counseling of patient before and after test

TEST - is a screening, NOT a diagnostics even it is more precise than conventional biochemical screening

- is giving no additional information about genome

- is not a part of routine prenatal genetic tests

Positive result of NIPT (chromosomal abnormality finding)

- genetic counseling of patient
- confirmation of result by amniocentesis

Negative results of NIPT (normal finding)

- false negative result possible
- it doesn´t mean automatically a healthy child

High risk population of pregnant women

- it is possible to offer the test, but then confirmation of abnormal result by amniocentesis

Therapy of genetic disorders

Metabolic manipulation

Dietary restriction

- *Lactose restriction for lactase deficiency; phenylalanine restriction for phenylketonuria*

Dietary supplementation

- *Vitamine C for scurvey; biotin for biotinidase deficiency; starch for G6PD defficiency*

Chelation and enhanced excretion/remove of excess of stored compound

- *Copper chelation for Wilson disease*
- *Periodic bleeding in hemochromatosis – removal of iron*

Metabolic inhibitors

- *Statins for hypercholesterolemia*

Therapy of genetic disorders

Gene product replacement

Hormone, protein or enzyme replacement or to increase its activity

- Hormone supplementation:

- *Hypothyroidism: thyroid*
- *Congenital adrenal hyperplasia: cortisol*
- *Turner syndrome: growth hormone*
- *Haemophilia: clotting factor*
- *Idiopatic male infertility: FSH treatment based on genotype*
- *Trombophilia: low molecular weight heparine intake based on genotype*

Insulin

- *Diabetes*

Enzyme replacement

- *Gaucher disease: beta glucosidase (Ceredase, Cerenzyme)*
- *Pompe disease: alpha glucosidase*
- *Hunter syndrome: iduronate-2 sulphatase (Elaprase)*
- *Fabry disease: alpha galactosidase A (Fabrazyme)*

Therapy of genetic disorders

Surgery
Cell and organ transplantation

Surgery

- *Cleft lip/palate; polydactylia, syndactylia: surgical reconstruction*
- *Breast cancer, colon cancer: removal of part of body or part ofthe organ*
- *Female infertility: personalized embryo transfer in IVF treatment* ***(genomic approach)***

Bone marrow transplantation

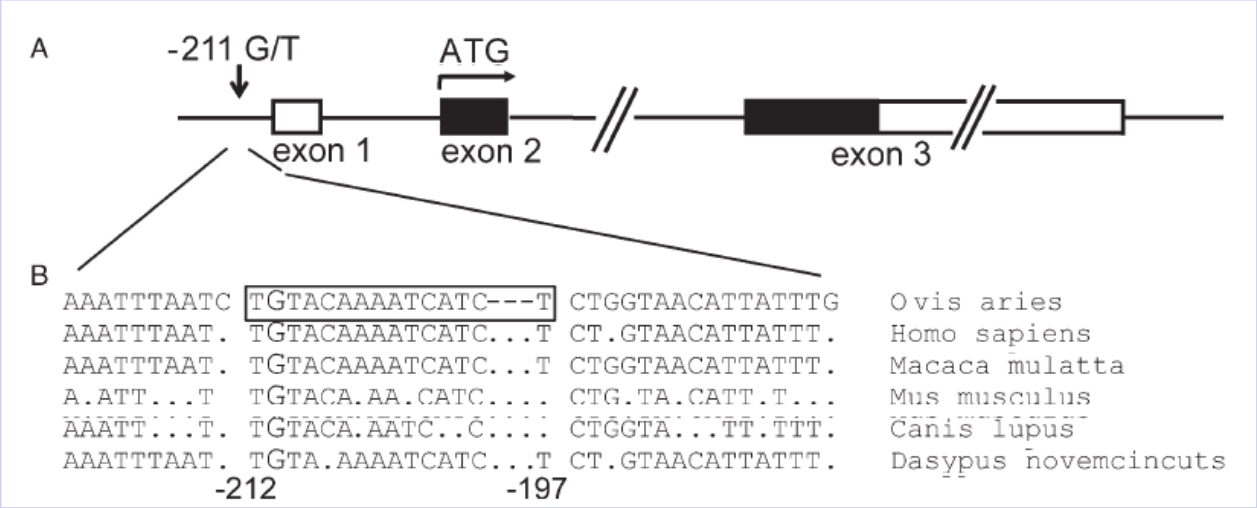
- *Thalasemia*
- *Chronic myeloid leukemia haemophilia*

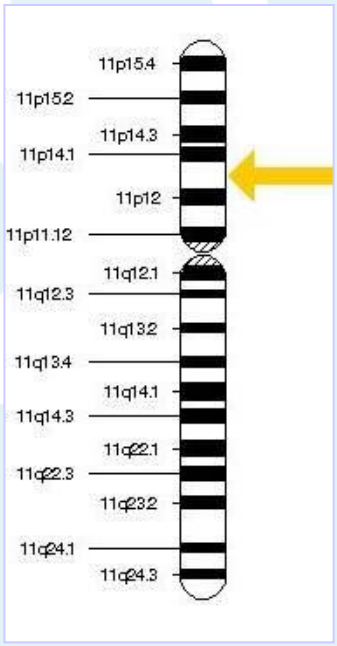
Controlling of environmental factors

- *Neural tube defects and myelomenigocele: folic acid administration in pregnant mothers*
- *Cardiovascular disease, diabetes type II, colon cancer: nutritional/lifestyle management*

Male idiopatic infertility

FSHB – gene encoding β-subunit of FSH





Folicule stimulating hormone (FSH) is produced by adenohypophysis – central hormone of human reproduction, which is essential for development of gonads and production of gametes

FSH stimulates the growth of folicules and oocytes in ovaries and Sertolli cells in testes and supports the production of sperm

Variants in promotor of FSHB gene

FSHB gene promotor : **transcription control of FSHB gene**

SNP rs10835638 .... nucleotide substitution **-211G>T**

Standard allele G **derived allele T** (20 – 25% of USA and Europe population)

Function of derived allele T:

significant decrease of transcriptional activity of FSHB gene

Genotypes:

-211G/-211G .... **Normal homozygote**

-211G/-211T .... **Heterozygote .... - 25% of activity of FSHB gene**

-211T/-211T .... **Homozygote for derived allele T**

.... **- 50% of activity of FSHB gene** ***(lower serum level of FSH)***

.... Oligozoospermia, lower volume of testes, decreased level of testosterone and higher level of LH in sera

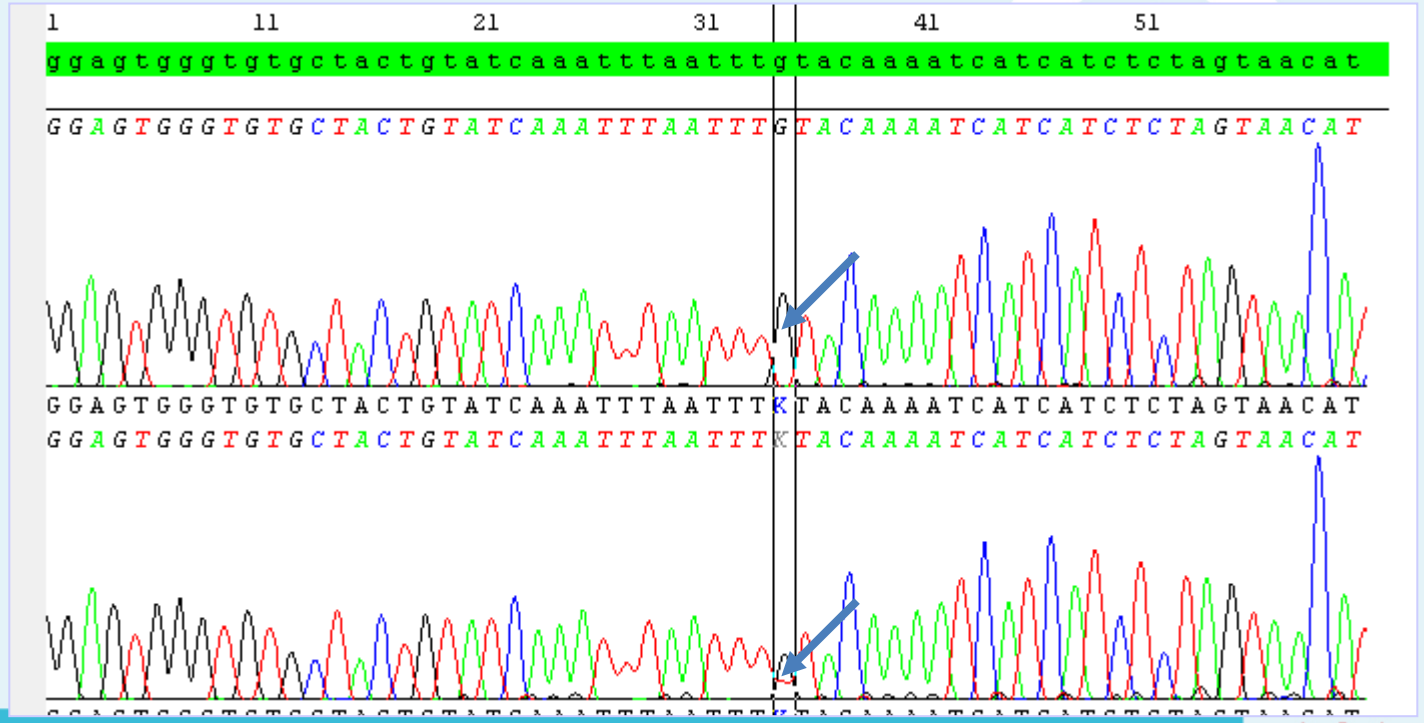
Variants in FSHB gene promotor

```
  1  GGAGCCAGAT CATGAAATGT TTTCTCTTTG TTTGTTTCTT CCTTCACAGC TTTTGATATG CTCTTGGAGC AATTTATTAA CCATATTTTT TAATGCATCT
     CCTCGGTCTA GTACTTTACA AAAGAGAAAC AAACAAAGAA GGAAGTGTCG AAAACTATAC GAGAACCTCG TTAAATAATT GGTATAAAAA ATTACGTAGA

101  CCTGAACAGA GTCAAAGCAA TACTTGGAAA GGACTCTGAA TTTCCTGATT TAAAGATACA AAAGAAAAAT CTGGAGTCAC AATTAATTTG AGAAGGTAAA
     GGACTTGTCT CAGTTTCGTT ATGAACCTTT CCTGAGACTT AAAGGACTAA ATTTCTATGT TTTCTTTTTA GACCTCAGTG TTAATTAAAC TCTTCCATTT

                                        PisaI
                                        TatI
201  GGAGTGGGTG TGCTACTGTA TCAAATTTAA TTTGTACAAA ATCATCATCT CTAGTAACAT TATTTTTTCT AATCTACTGC GTTTAGACTA CTTTAGTAAA
     CCTCACCCAC ACGATGACAT AGTTTAAATT AAACATGTTT TAGTAGTAGA GATCATTGTA ATAAAAAAGA TTAGATGACG CAAATCTGAT GAAATCATTT

301  GCTTGATCTC CCTGTCTATC TAAACACTGA TTCACTTACA GCAAGCTTCA GGCTAGCATT GGTC
     CGAACTAGAG GGACAGATAG ATTTGTGACT AAGTGAATGT CGTTCGAAGT CCGATCGTAA CCAG
```

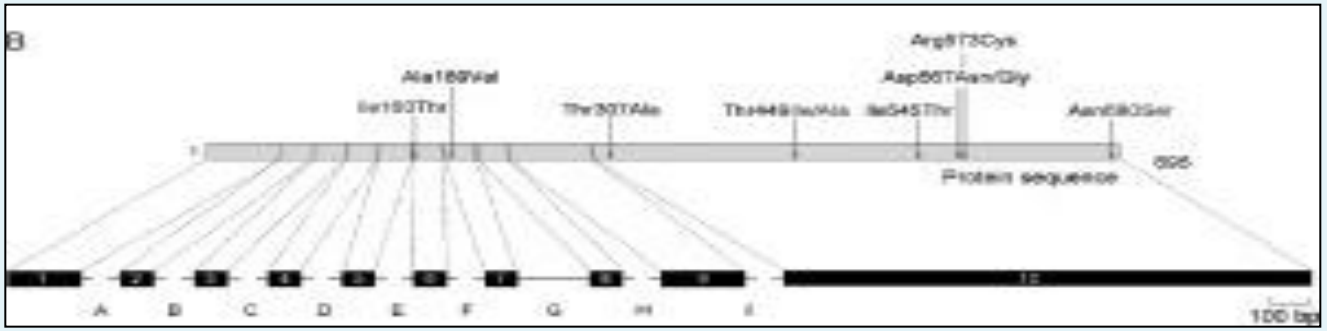
Genotype:

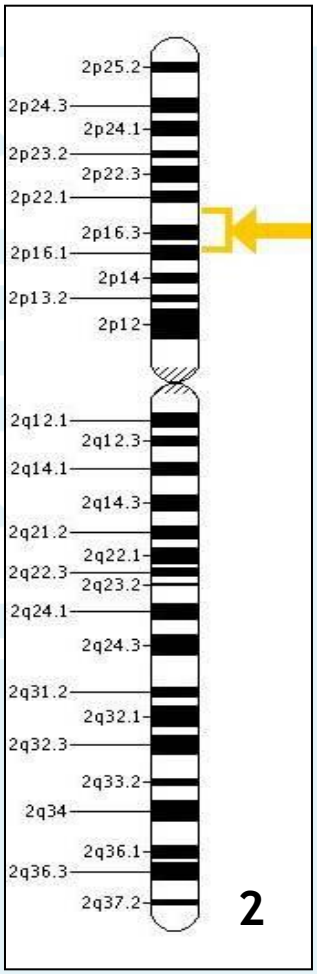
homozygote G/G

heterozygote G/T



FSHR – FSH receptor gene





FSHR gene promotor : **SNP rs1394205** .... **-29G>A**

Dominant , occurence independent on SNP in exon 10

Exon 10 of FSHR gene: **SNP rs6165** .... c.919G>A .... 307: Thr/Ala
SNP rs6166 .... c.2039A>G .... 680: Asn/Ser

- genetic linkage

Variants in promotor of FSHR gene

FSHR gene promotor: **transcription control of FSHR gene**

SNP rs1394205 .... nucleotide substitution **-29G>A**

Standard allele G **derived allele A**

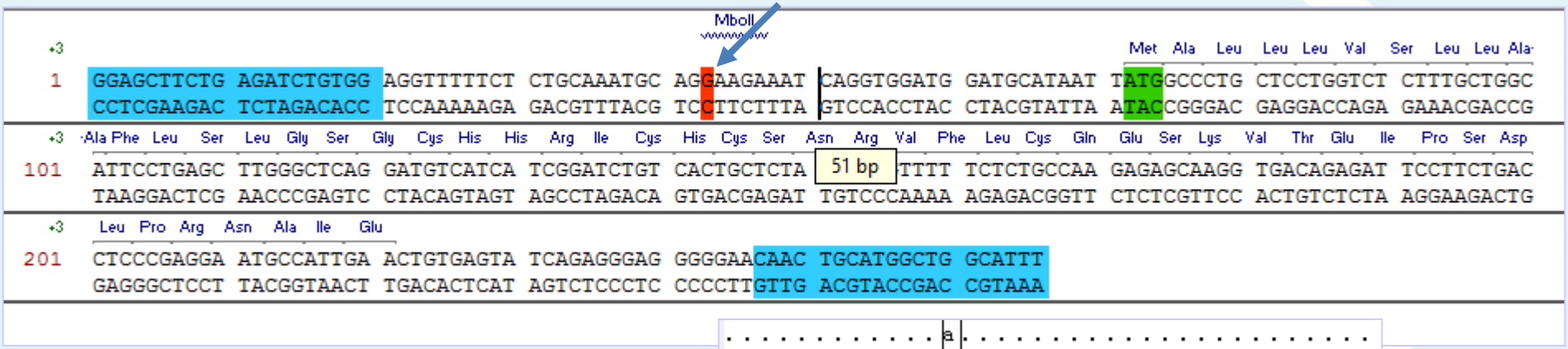
Function of derived allele:

significant decrease of transcriptional activity of FSHB gene

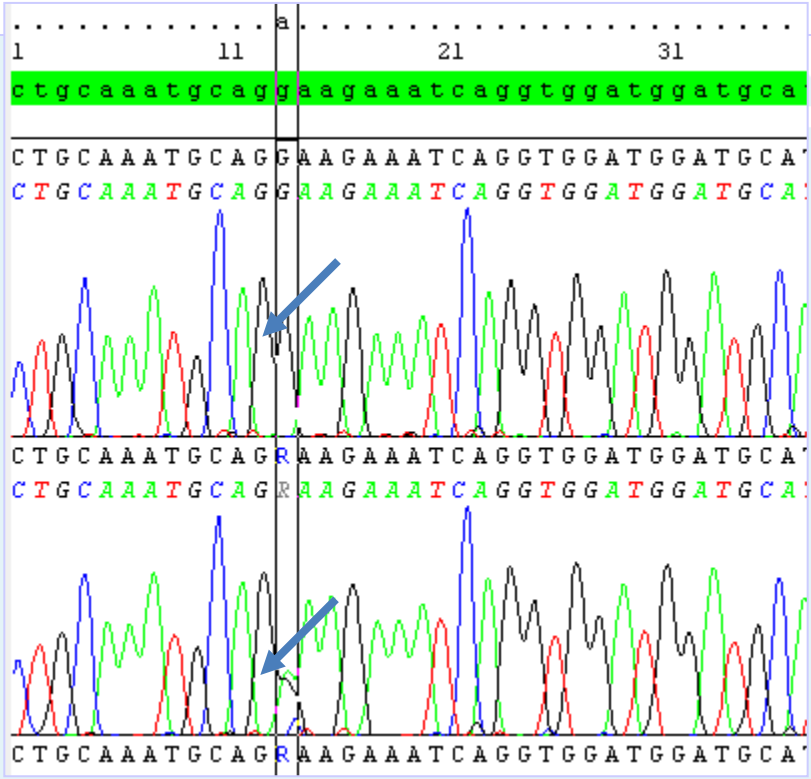
Genotype:

-211G/-211G	**....**	**Normal homozygote**
-211G/-211A	**....**	**Heterozygote .... - 30% of activity of FSHB gene**
-211A/-211A	**....**	**Homozygote for derived allele T**
	....	**- 56% of activity of FSHR gene**

Analysis of FSHR -29G>A (rs1394205) using SNA sequencing



Genotype: homozygote G/G



heterozygote G/A

Variants in exon 10 of FSHR gene

SNP rs6165 .... nucleotide substitution **c.919G>A** .... **307: Thr/Ala**

Standard allele G **derivoved allele A**

SNP rs6166 .... nucleotide substitution **c.919G>A** .... 680**: Asn/Ser**

Standard allele A **derivoved allele G**

Function of derived allele:

- **different sensitivity of receptor to FSH hormone according to genotype**
- **different response to exogenous injection of FSH according to genotype**

Variants in exon 10 of FSHR gene

Genotypes:

Thr307Asn680/Thr307Asn680 .... **Normal homozygote**

Thr307Asn680/Ala307Ser680 .... **Heterozygote**

.... Medium decrease sensitivity of receptor to FSH

Ala307Ser680/Ala307Ser680 .... **Mutated homozygote (40%)**

.... Higher decrease of sensitivity of receptor to FSH

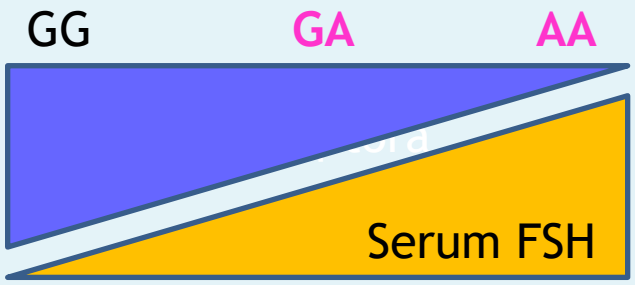
Recombinant genotypes

Ala307Asn680 (1%)

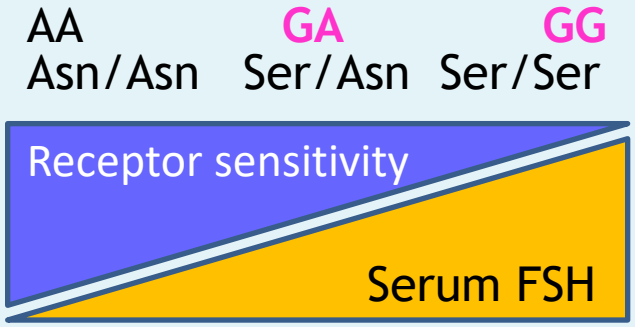
Thr307Ser680 (1%)

Impact on FSH level

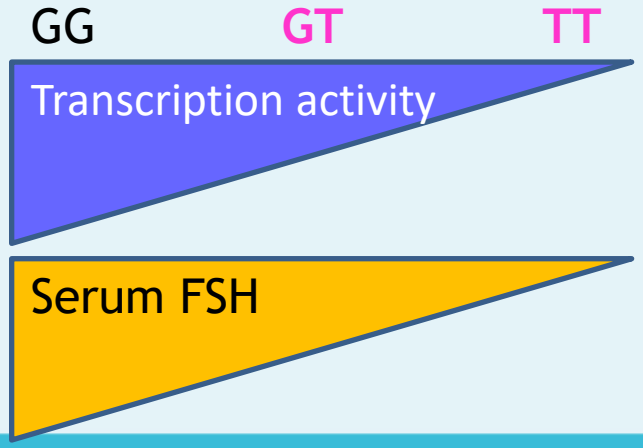
FSHR -29G>A (promotor)



FSHR p.N680S (exon 10)



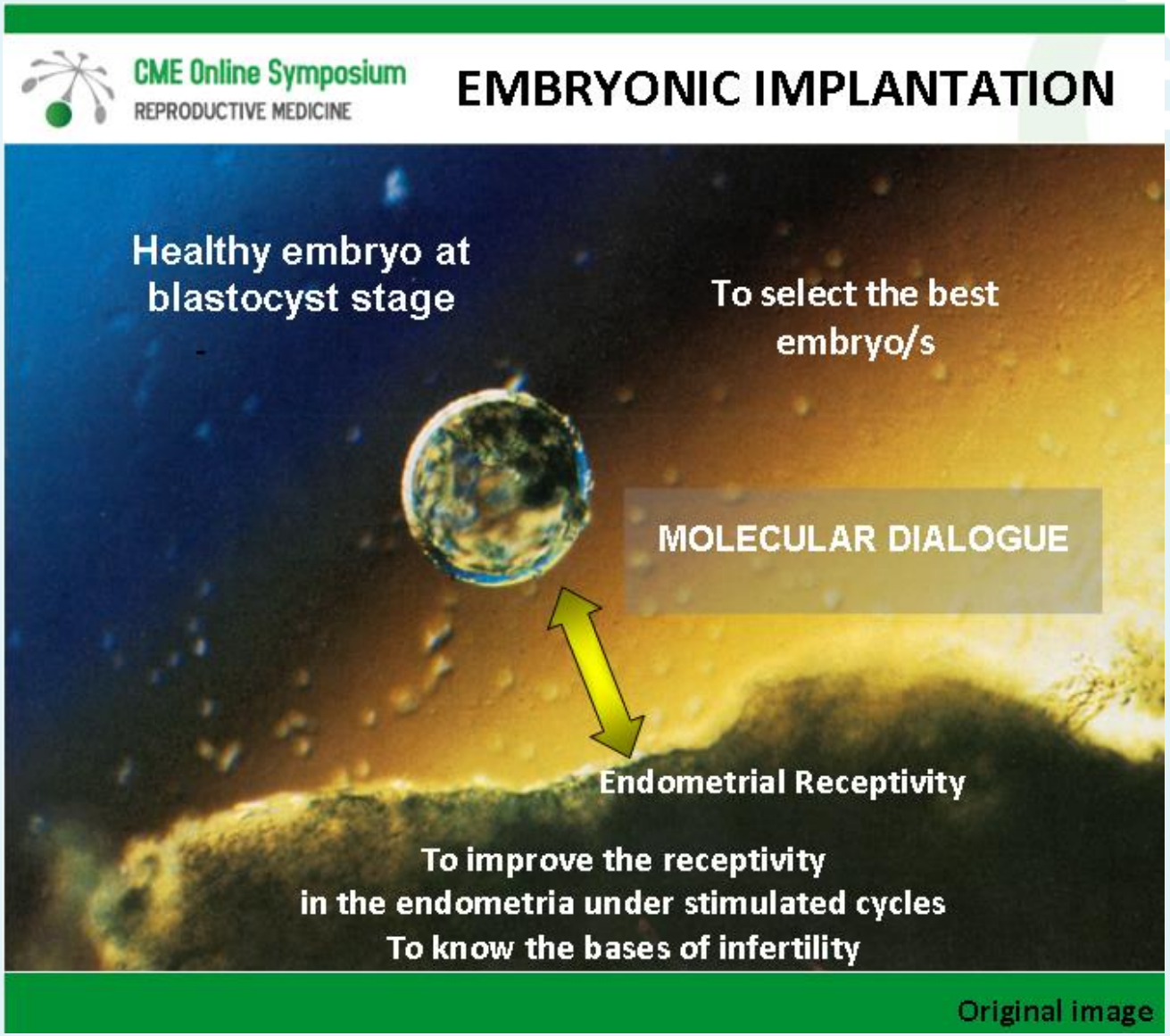
FSHB -211G>T (promotor)



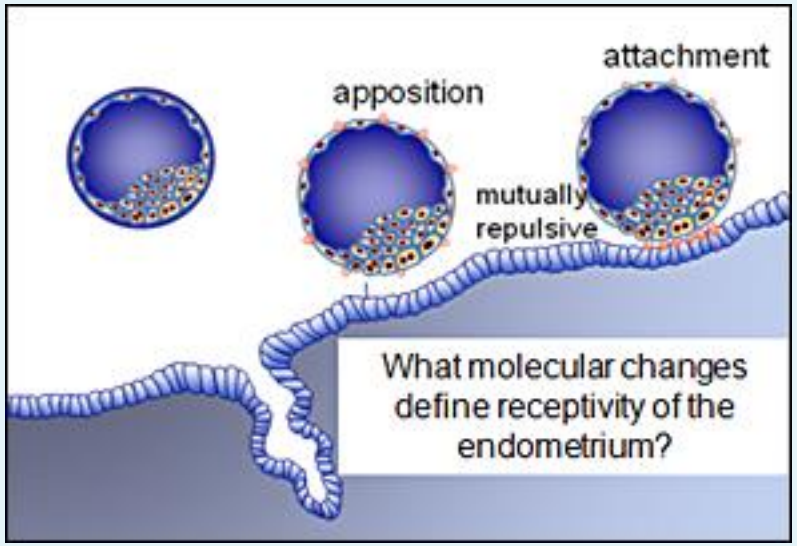
Treatment by FSH - 12 weeks

(prof. M. Simoni, 2014)

ERA® ENDOMETRIAL RECEPTIVITY ASSAY



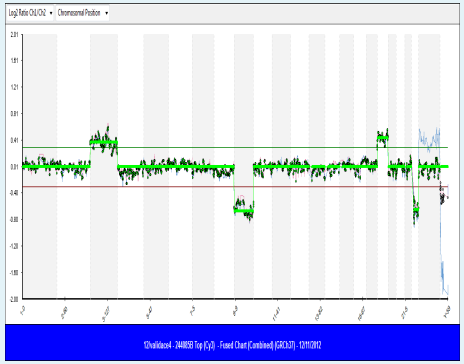
ERA® ENDOMETRIAL RECEPTIVITY ASSAY



24% of infertile women with repeated implantation failure has changed implantation window

(delayed or advanced)

even good quality of embryos are transfered



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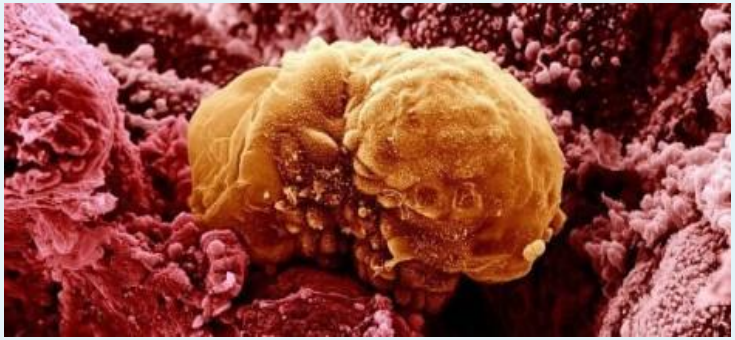
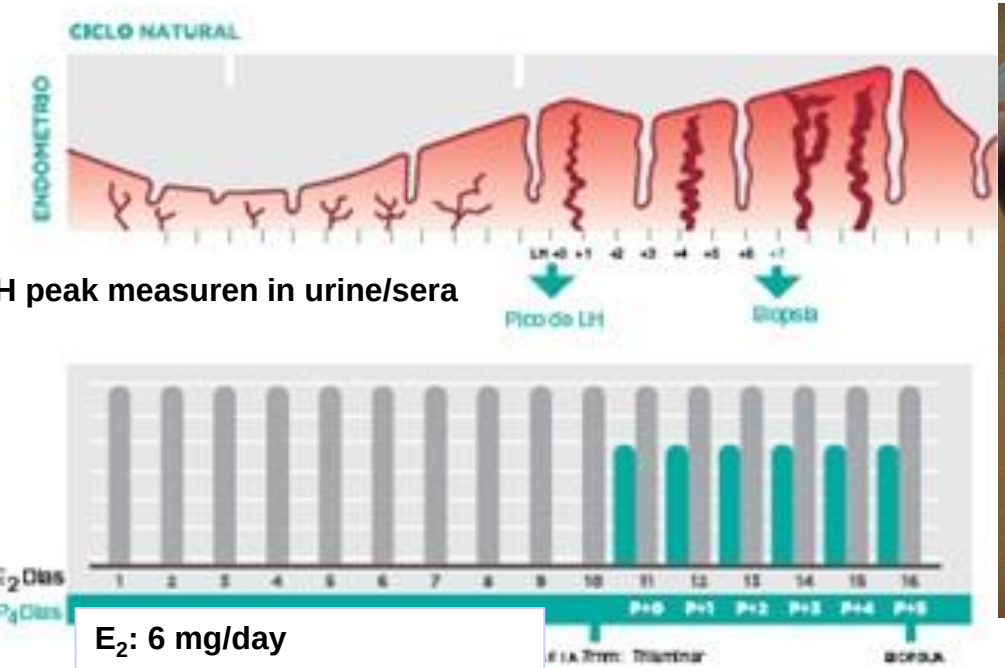


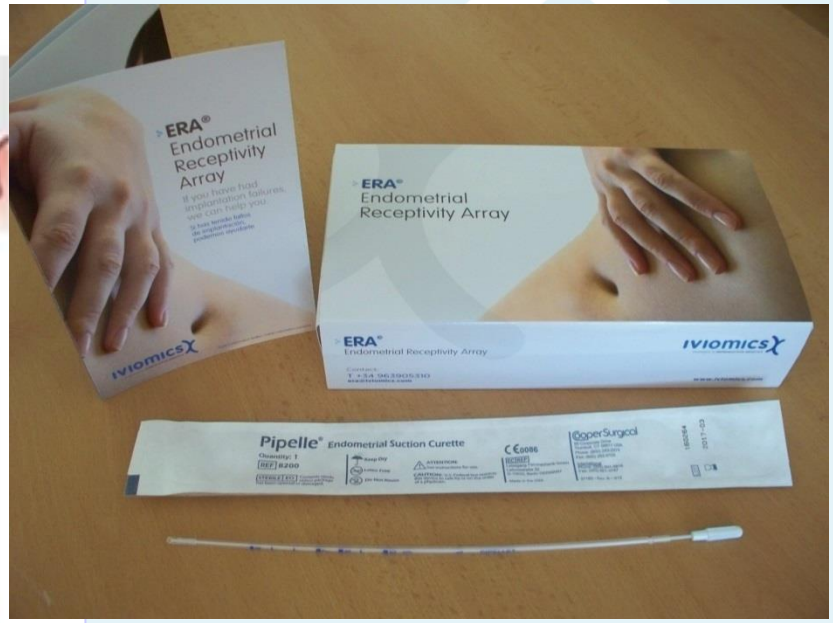
ERA® ENDOMETRIAL RECEPTIVITY ASSAY



LH peak measuren in urine/sera

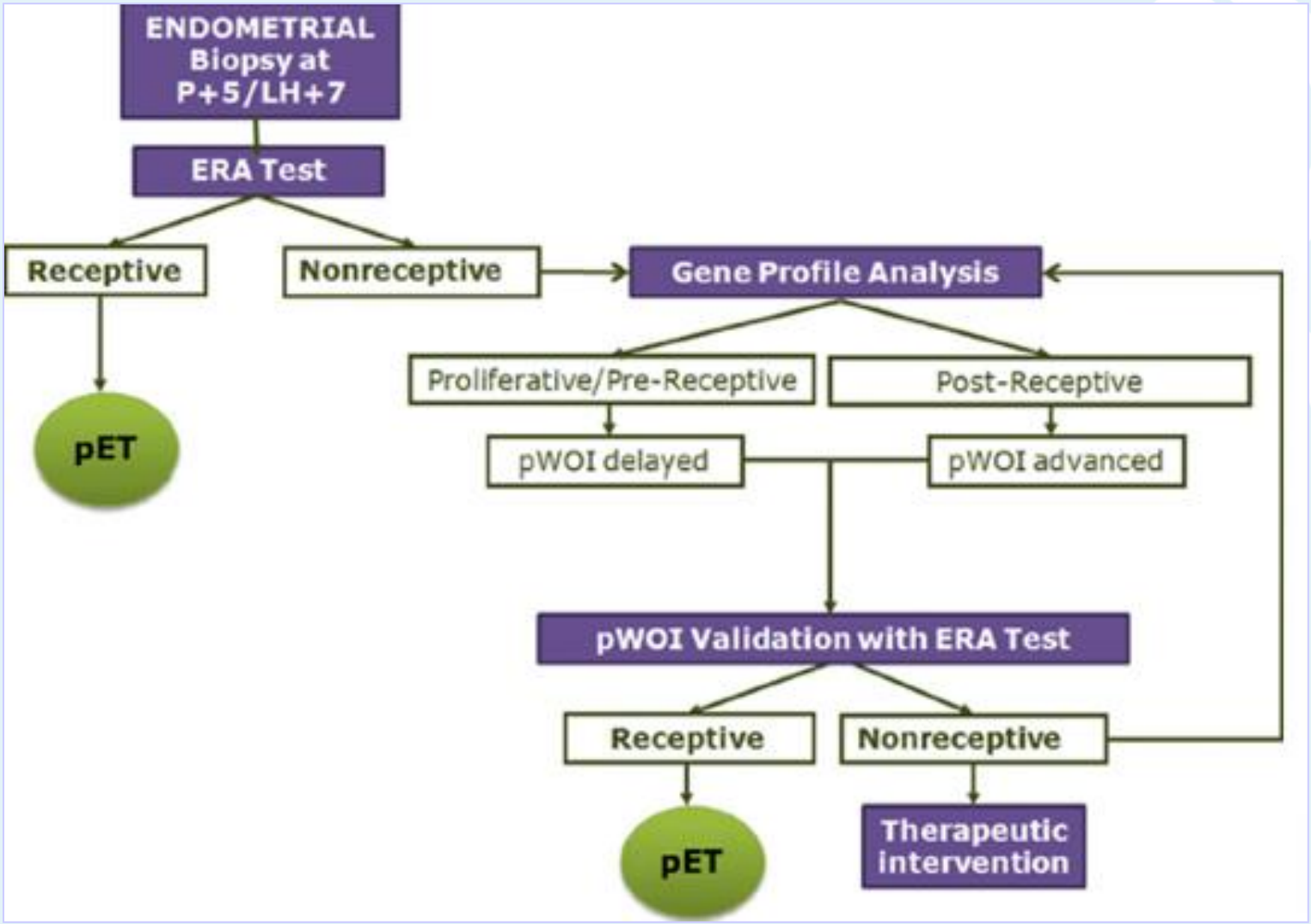
E_2: 6 mg/day

E_4: 800 mg/day

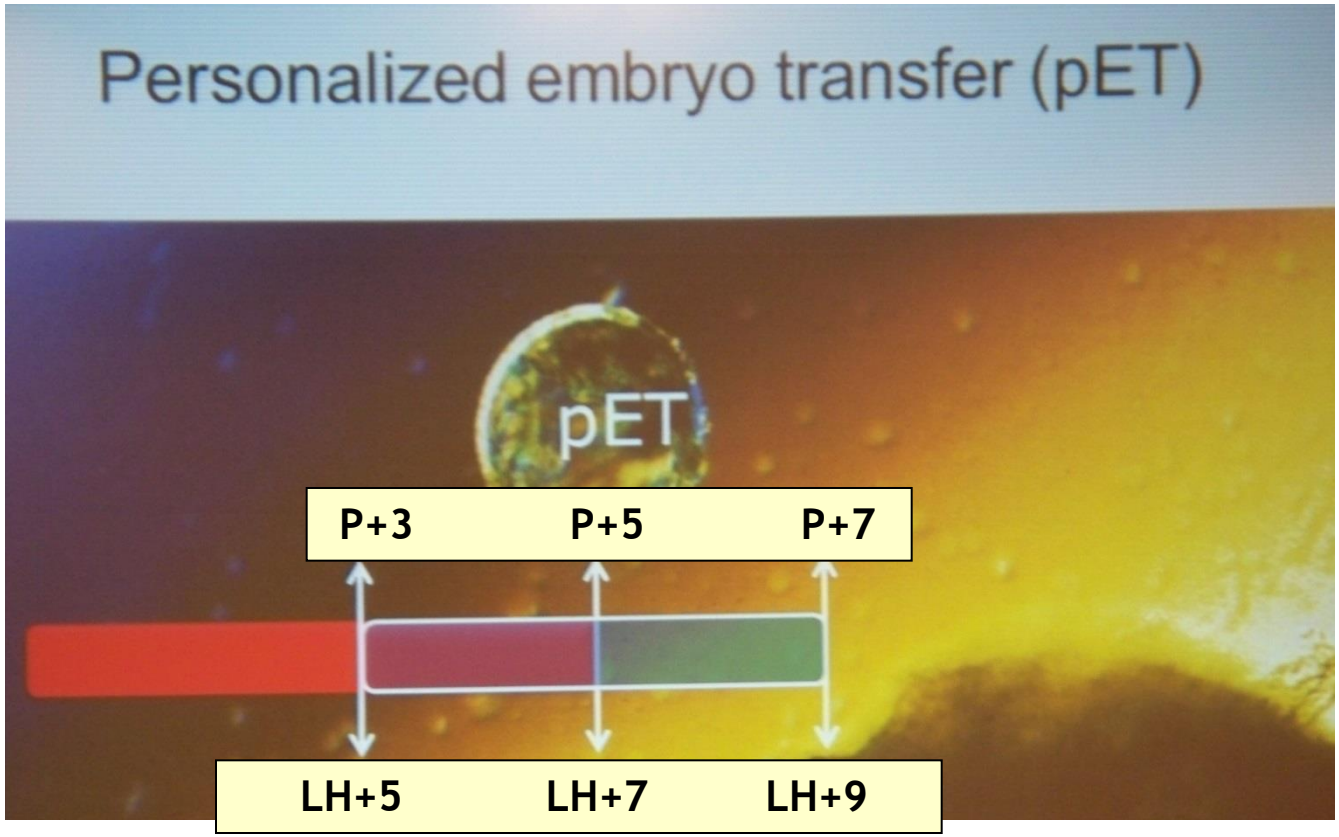


- **Molecular customer-made microarray prepared for diagnostics of endometrial receptivity in IVF treatment**
- **Examination of expression of 238 genes participating in receptivity of endometrium**
- **Classification of endometrium as „receptive“ or „non-receptive“**

Algorhytm of personalized embryo trasfer



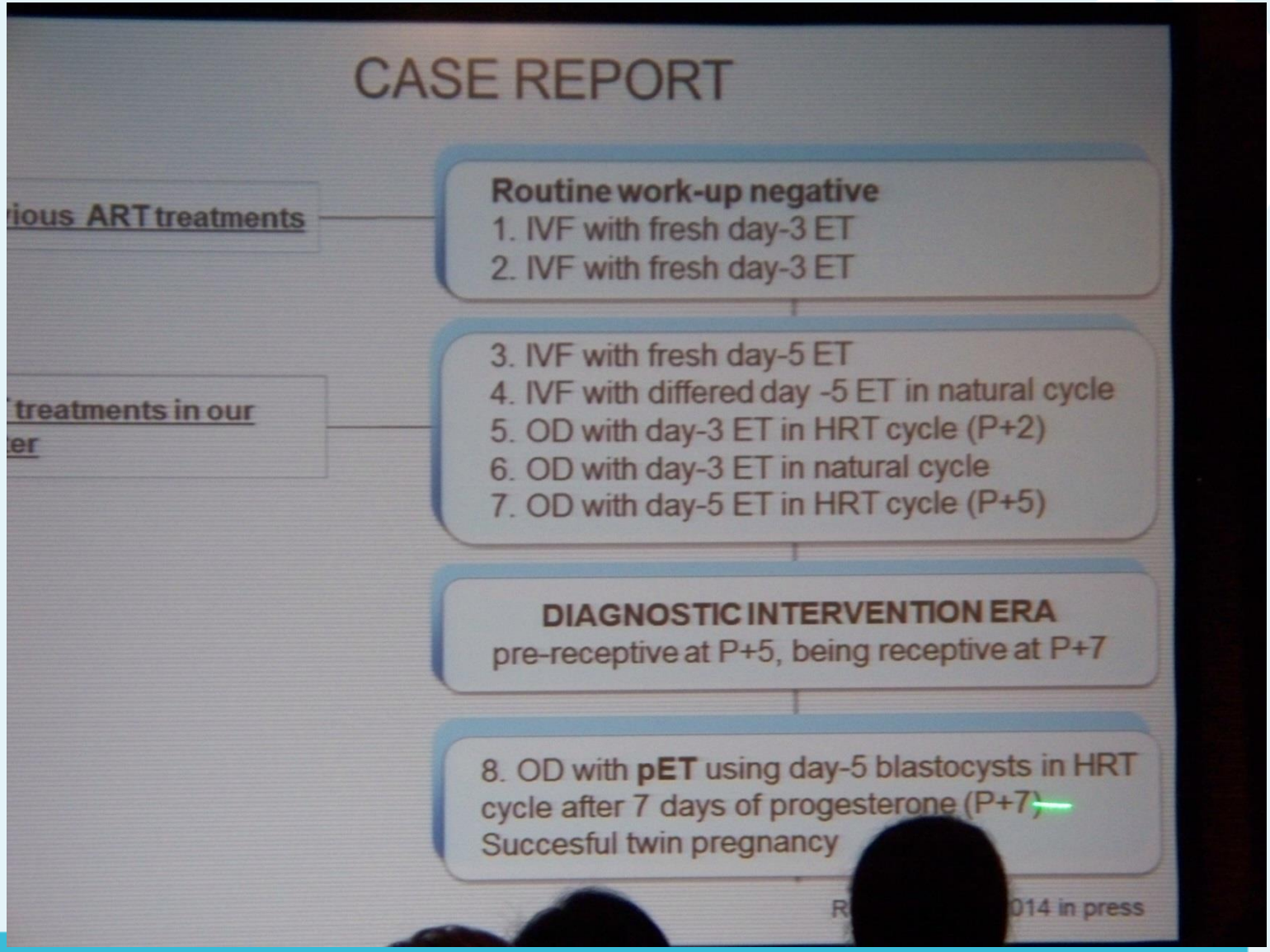
Personalized embryo transfer



ET

pET advanced

pET delayed


CASE REPORT
ious ART treatments
treatments in our
er
Routine work-up negative
1. IVF with fresh day-3 ET
2. IVF with fresh day-3 ET
3. IVF with fresh day-5 ET
4. IVF with differed day -5 ET in natural cycle
5. OD with day-3 ET in HRT cycle (P+2)
6. OD with day-3 ET in natural cycle
7. OD with day-5 ET in HRT cycle (P+5)
DIAGNOSTIC INTERVENTION ERA
pre-receptive at P+5, being receptive at P+7
8. OD with pET using day-5 blastocysts in HRT cycle after 7 days of progesterone (P+7)
Succesful twin pregnancy
014 in press

Diagnostic importance of ERA®

Diagnostics of endometrium during implantation window

Precise detection and timing of embryo transfer according to the molecular characteristics of particular woman

↓

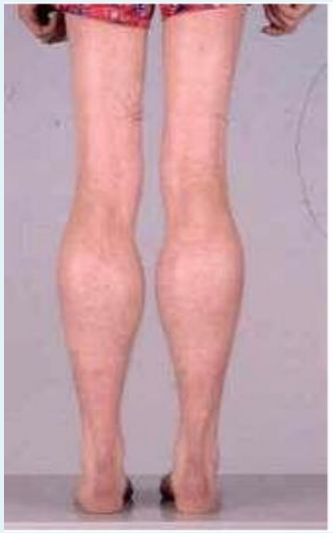
Personalized embryo transfer

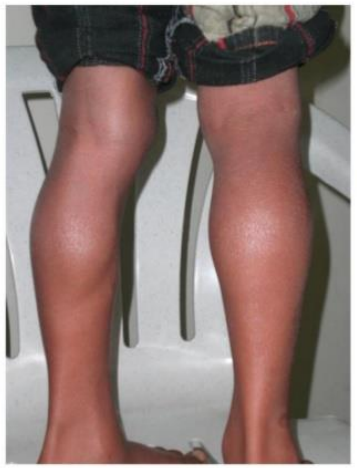
Genetic therapy

Duchenne muscular dystrophy classical form

- Progressive neuromuscular disorder
- Loss of walking, invalid at teenager age
- Death as consequence of cardiac or pulmonary failure
- Incidence 1 : 3 500 newborn boys
- Inheritance: X-linked recessive

  with symptomatic heterozygotes
- Cause of disorder: mutations in DMD gene





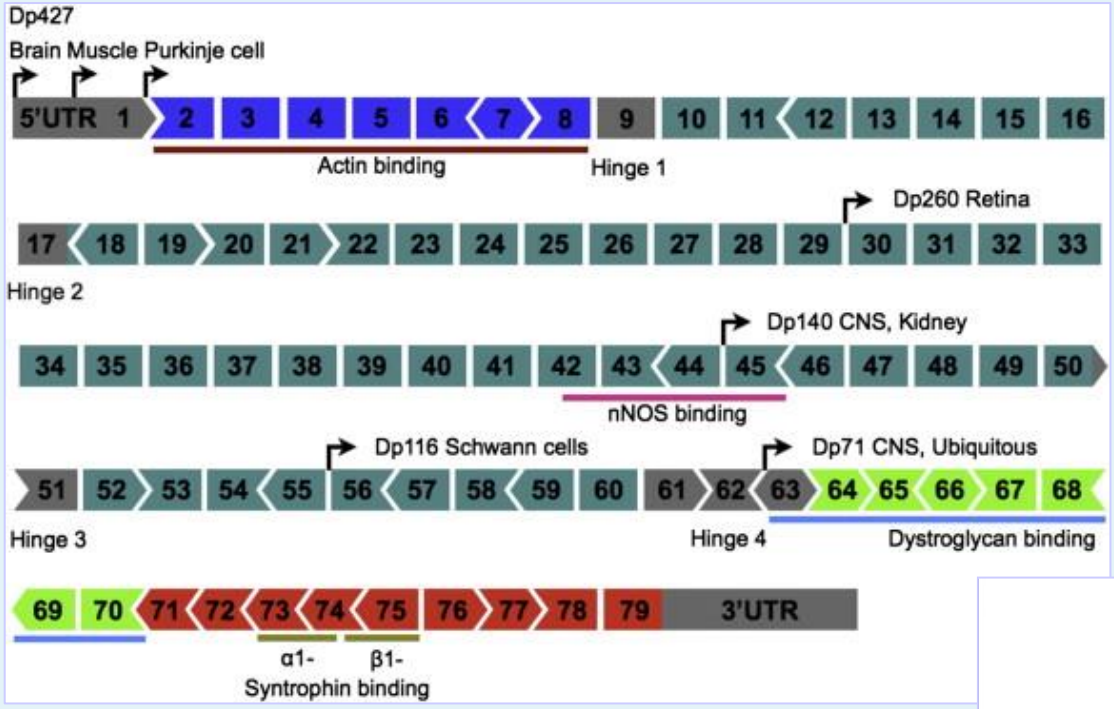
Fenotype variability of Duchenne muscular dystrophy

- **Becker muscular dystrofy**
  – late onset of symptoms, milder form, slower progression
- **Cardiomyopatic form**
  – minimal symptoms of skeletal muscles, dilatation form causing the cardiac arrhytmia and heart failure
- **DMD/BMD heterozygote women**
  – increased levels of creatinine kinase, muscle weakness, some symptoms of myocardiac damage (palpitation, stenocardia, dyspnoe), they need cardiologic dispensarization at adult age

Diagnostics of DMD

- Clinical presentation
- Elevation of serum creatin kinase (5-100x, ref.<2-4 **µkat/l**),
- Increased level of LD a transaminase enzymes
- EMG – severe myogene lesions
- DNA analysis – 97% of patients
- Biopsy ??

DMD gene encoding for dystrophin

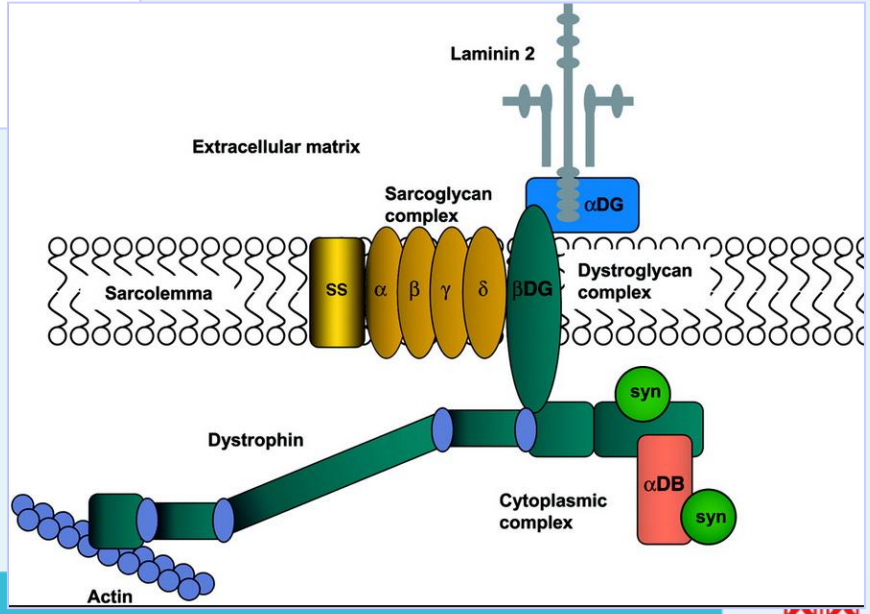


DMD gene has
2.300.000 nucleotides

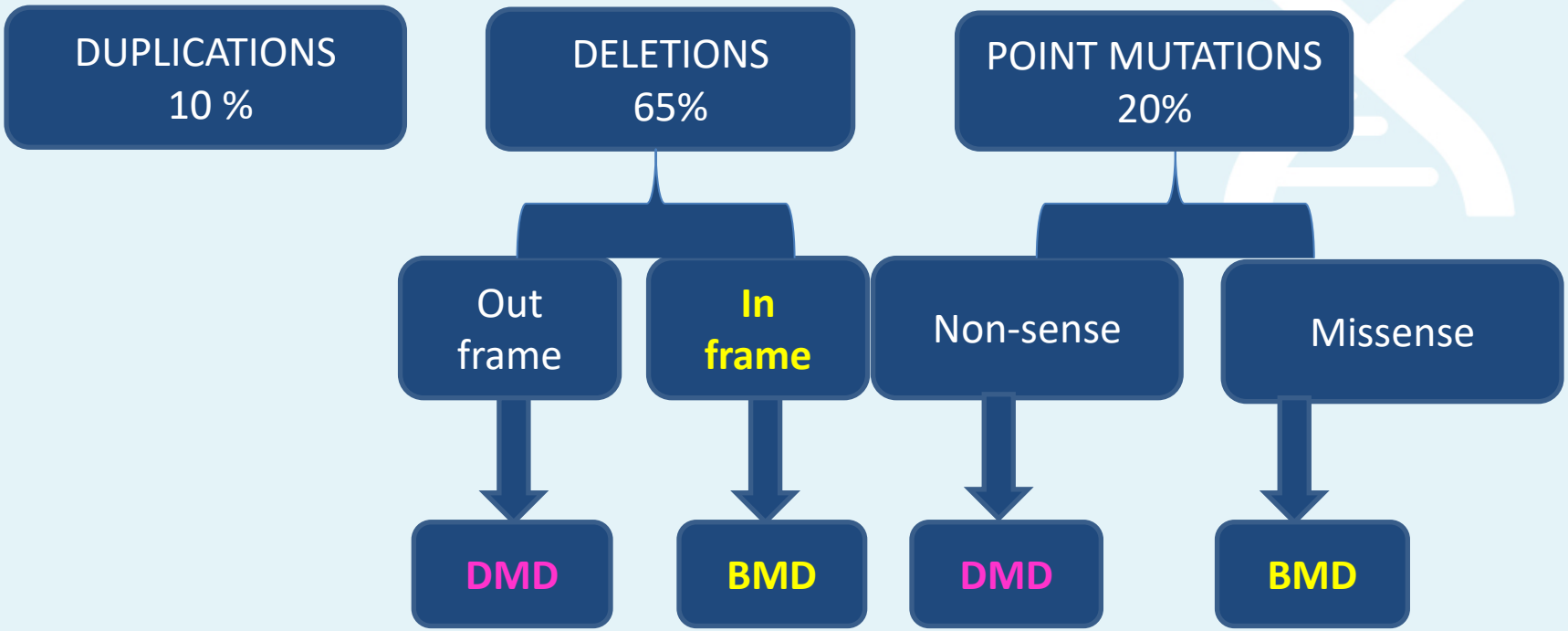
79 exons

Dystrofin – 3685 amino acids

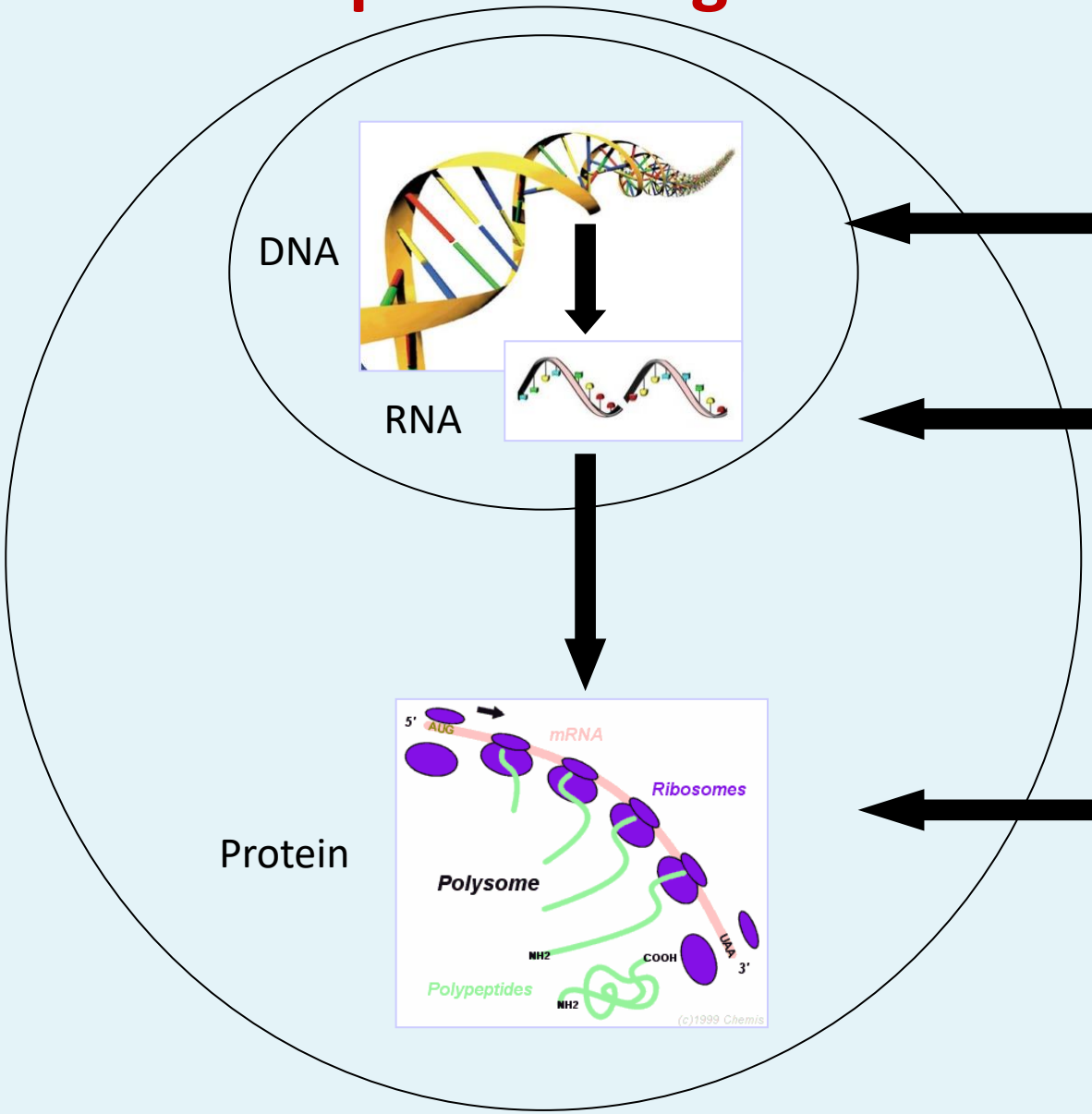
Dystrophin is participating in formation of cytoskeleton (it binds to actin and to signal molecules extracellular matrix – beta dystroglycan)



Mutations in DMD gene



The options of "genetic" therapy

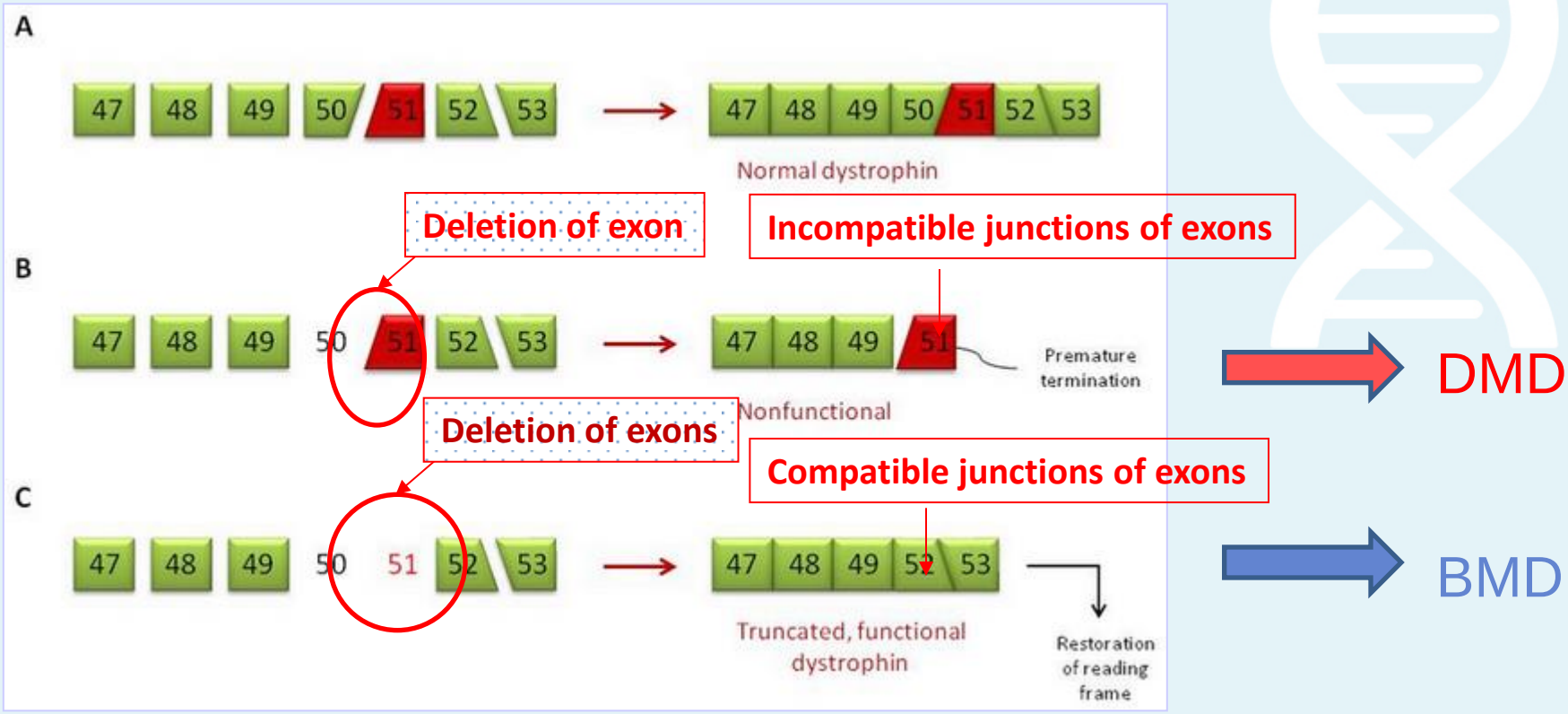


Gene therapy
- microdystrophin virus vector

Modication at the RNA level
- Antisense oligonucleotides exon-skipping

Modification of translation
- gentamycin
- PTC 124 (ataluren) Translarna

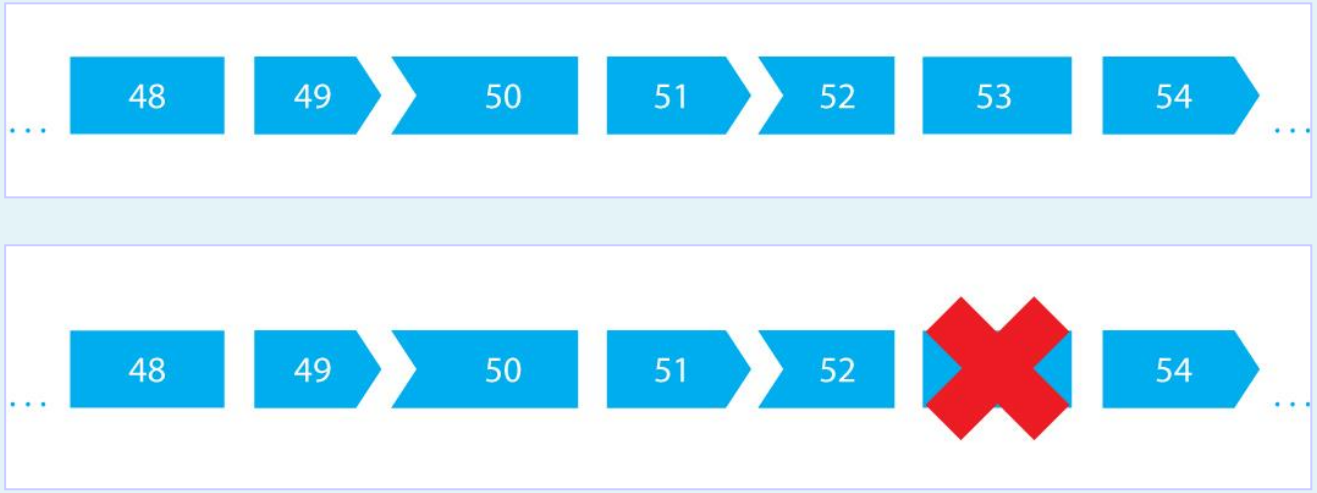
Deletions in DMD gene



Ryszard Kole:[Targeting mRNA Splicing as a Potential Treatment for Duchenne Muscular Dystrophy](), 2012.

What happens at Becker MD

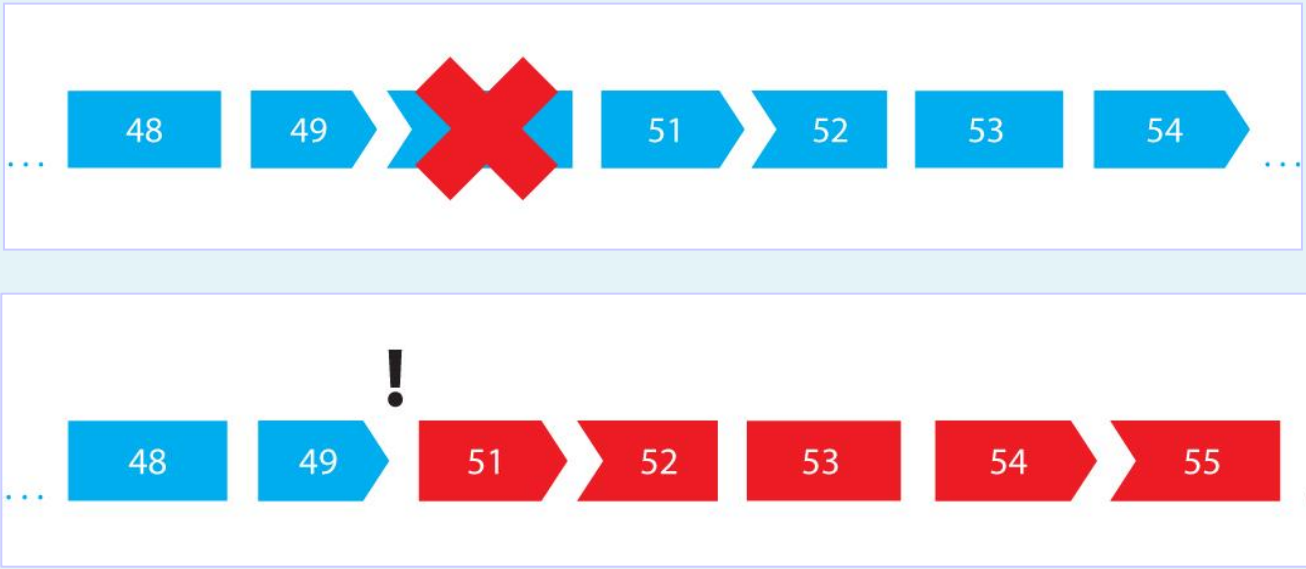
- Exons 48 – 54



- Part of gene is missing, but exon 52 can connect to exon 54 and all the rest of gene

http://www.musculardystrophyuk.org/progress-in-research/background-information/what-is-exon-skipping-and-how-does-it-work/

What happens at Duschenne MD?



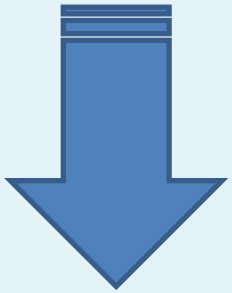
- The deletion of exon 50 is leading in **short protein, which lost its function** and in severe form of DMD – **exon 49 is not able to connect to exon 51**

http://www.musculardystrophyuk.org/progress-in-research/background-information/what-is-exon-skipping-and-how-does-it-work/

Exon-skipping

Goal : **To connect the exons to restore the open reading frame for translation**

DM DUCHENNE

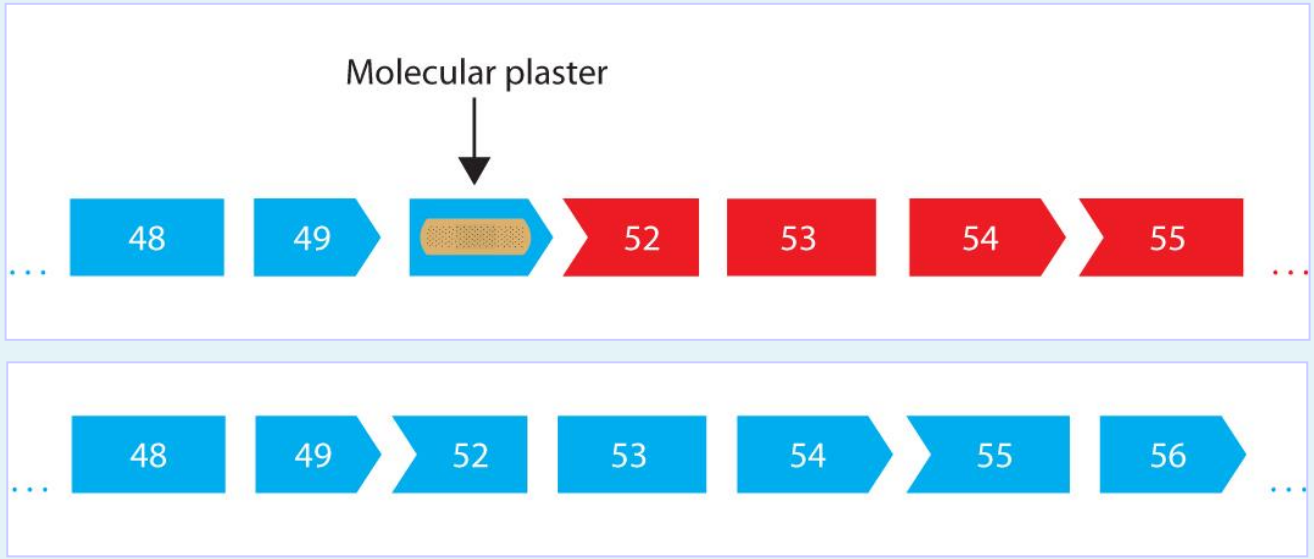


DM BECKER

http://www.musculardystrophyuk.org/progress-in-research/background-information/what-is-exon-skipping-and-how-does-it-work/

How can help us the exon-skipping?

- **AOs –antisense oligonucleotides or „molecular plasters“**
- **They mask exons and help to connect the exons with compatible ends**



http://www.musculardystrophyuk.org/progress-in-research/background-information/what-is-exon-skipping-and-how-does-it-work/

Development ...

- **EXON SKIPPING 51:**

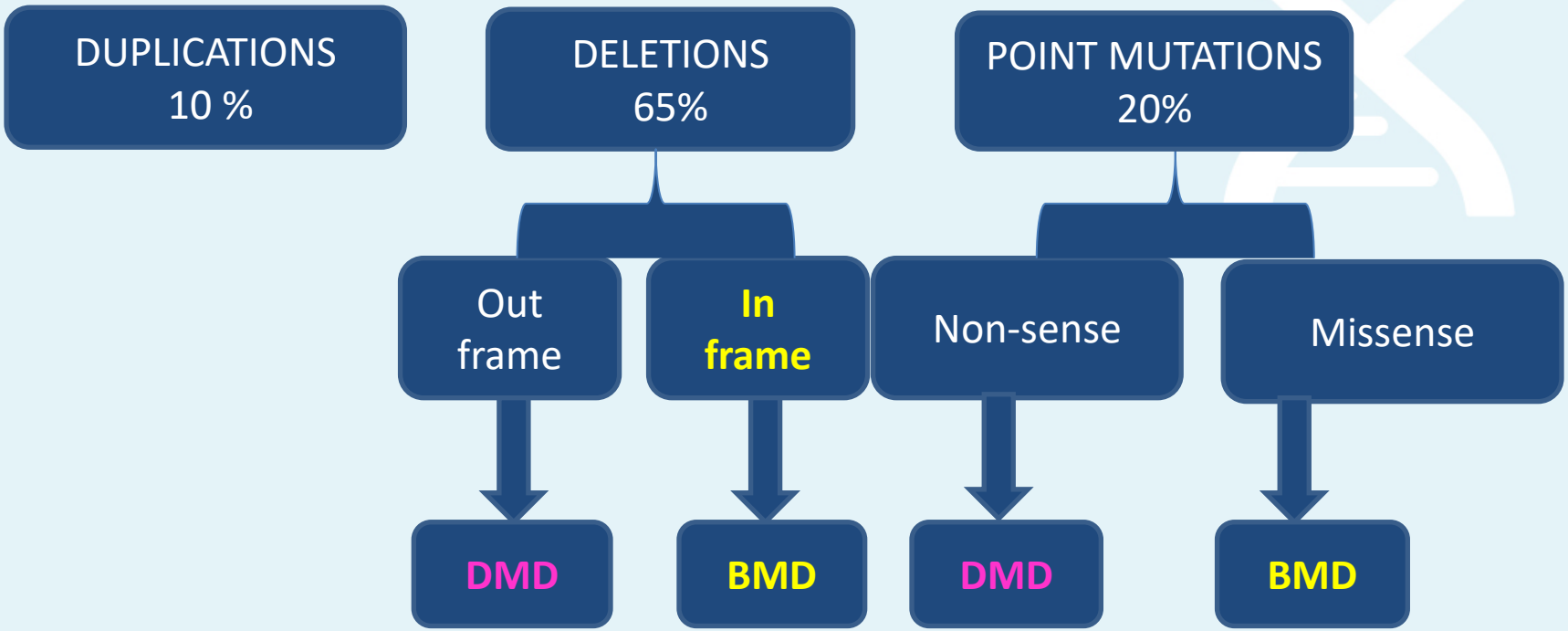
- **BIOMARINE - Drisapersen, Phase III**
- **SAREPTA – Eteplirsen, Phase III**

13% of DMD patients

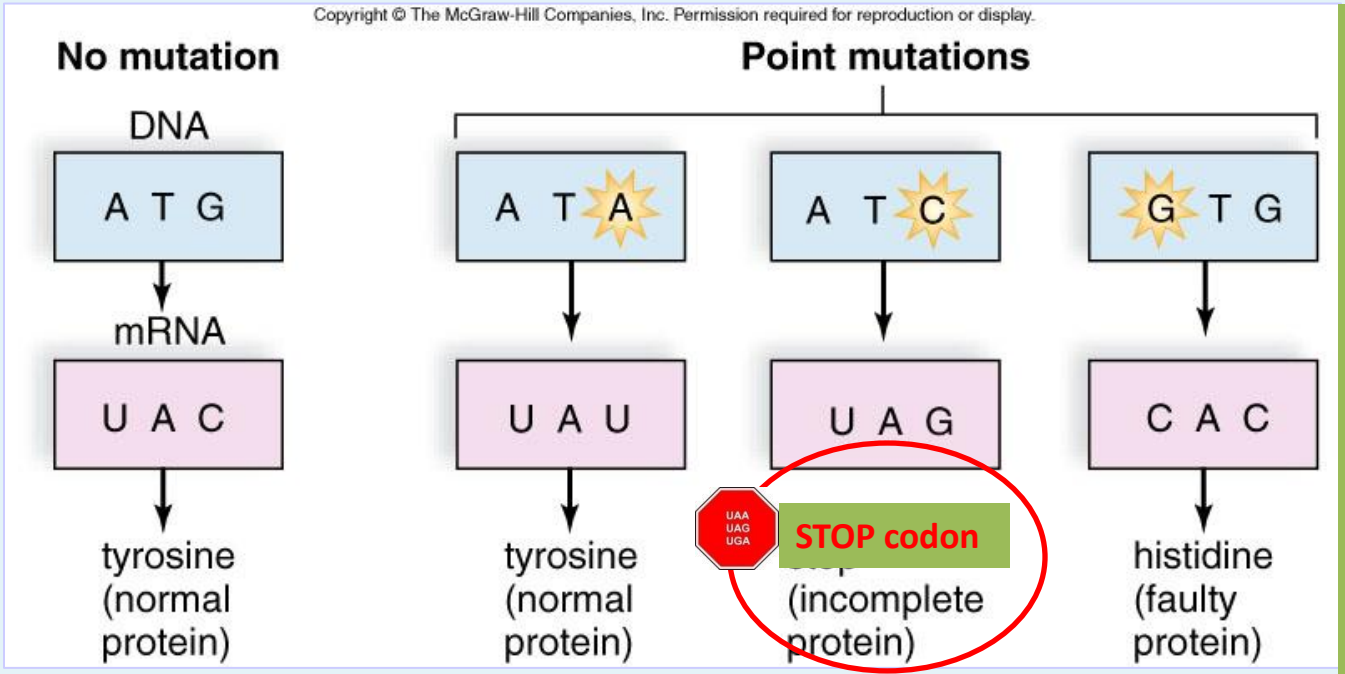
- **Process of registration by FDA, EMA ?**

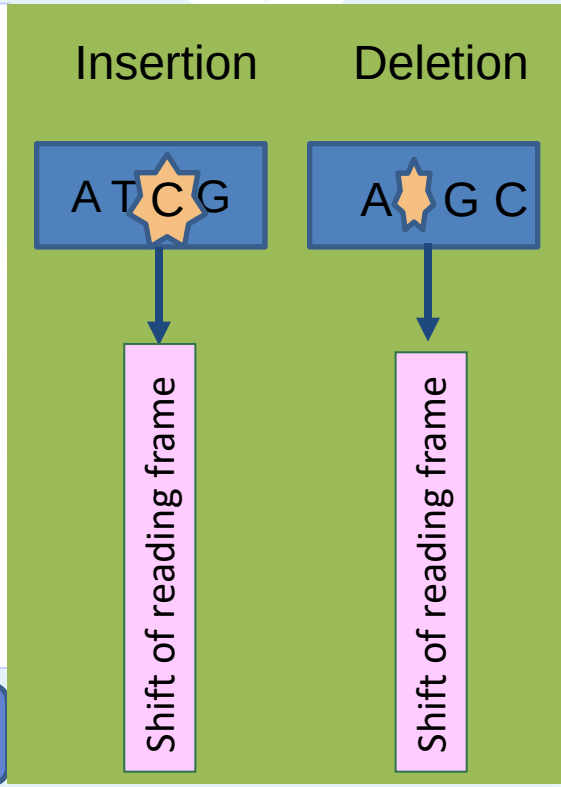
www.sarepta.com
www.biomarine.com

Mutations in DMD gene



Point mutations





Nonsense → nmDMD

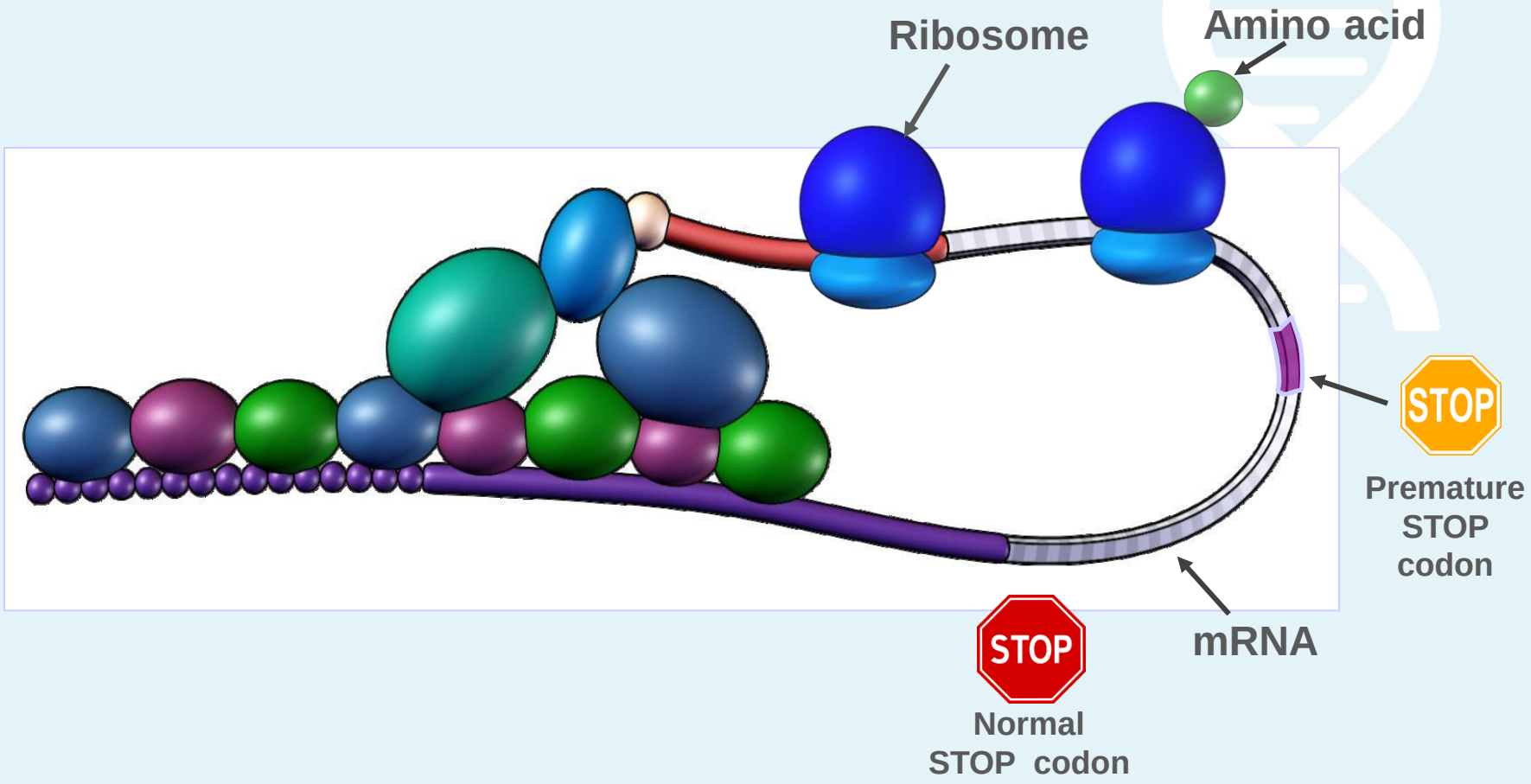
Missense → BMD

Shift of reading frame → DMD

Shift of reading frame → DMD

End of translation
Formation of incomplete, non-functional protein
Severe form of DMD

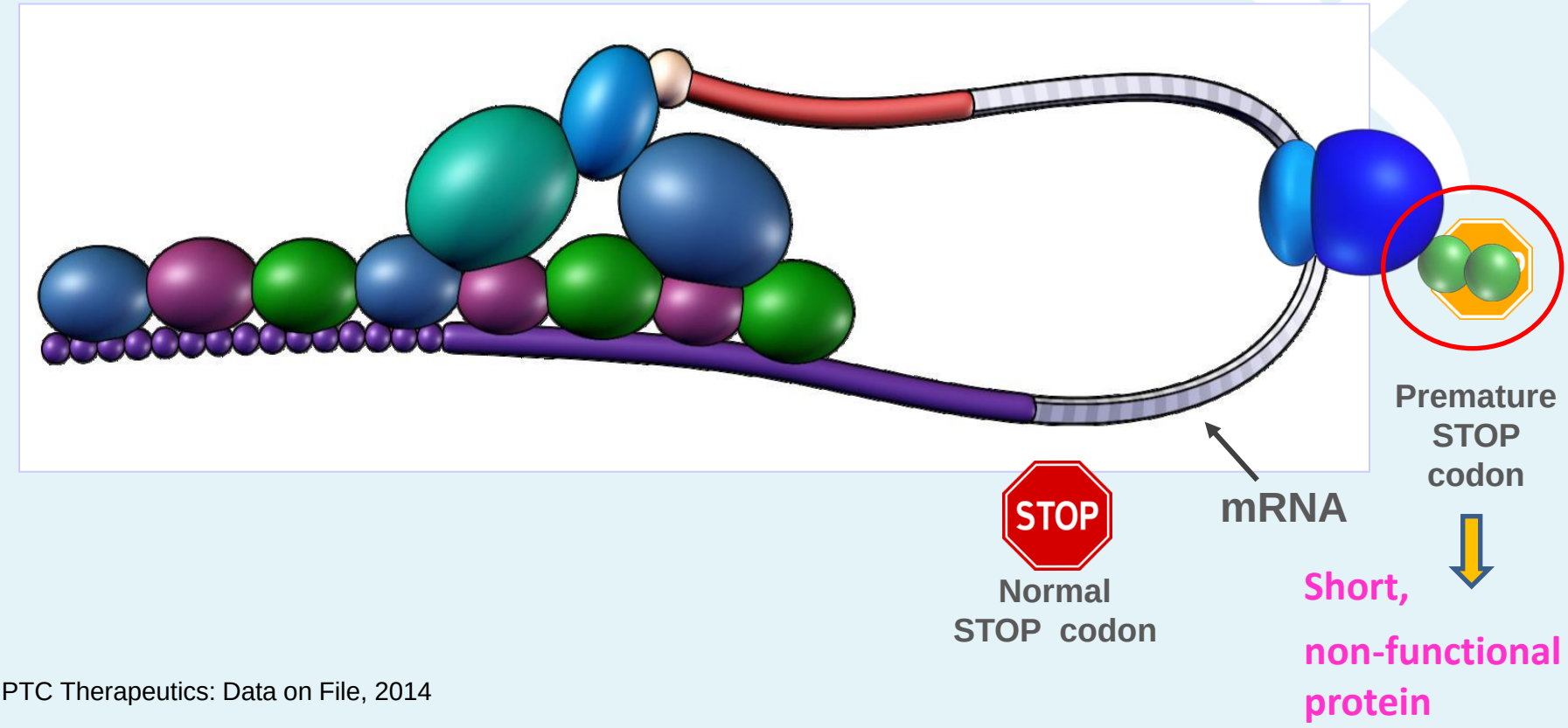
Non-sense mutation DMD/nmDMD



PTC Therapeutics: Data on File, 2014

Non-sense mutation DMD/nmDMD

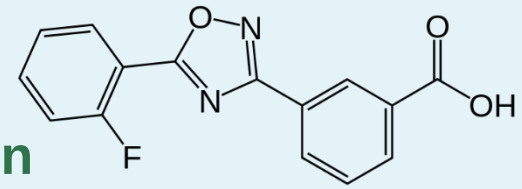
Premature STOP codon results in premature end of translation and in short, non-functional protein



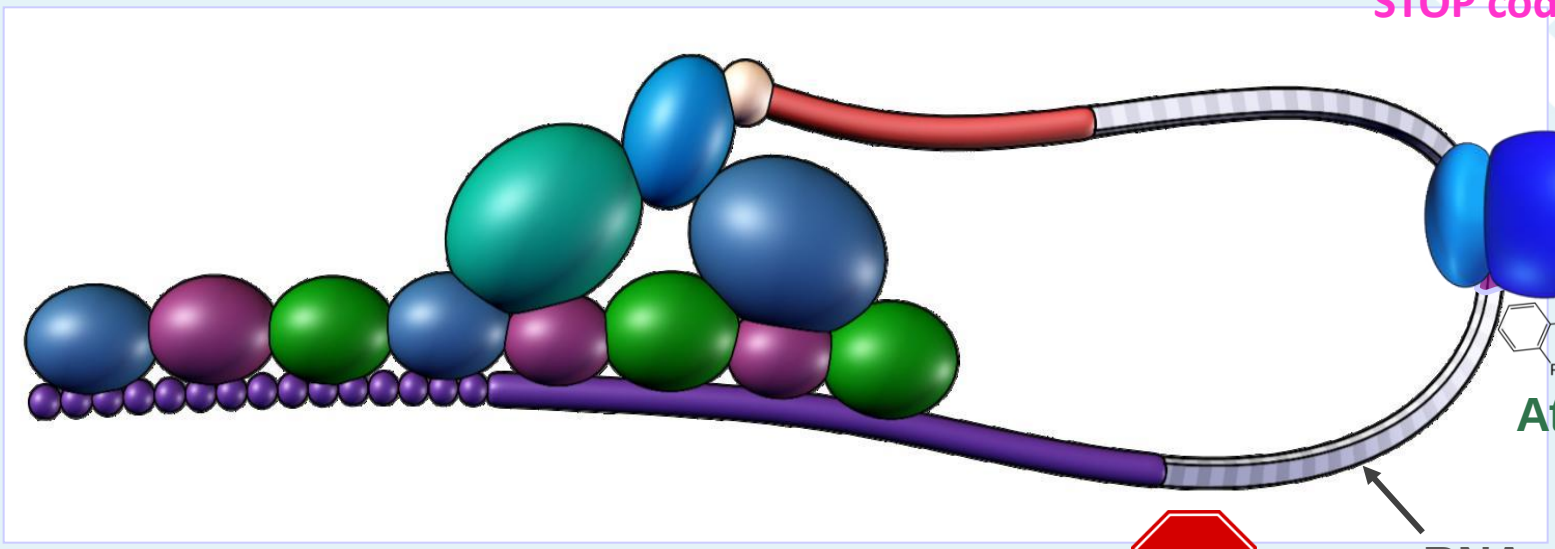
PTC Therapeutics: Data on File, 2014

ATALUREN – read through molecule

Ataluren



Ataluren is able to read the premature STOP codon



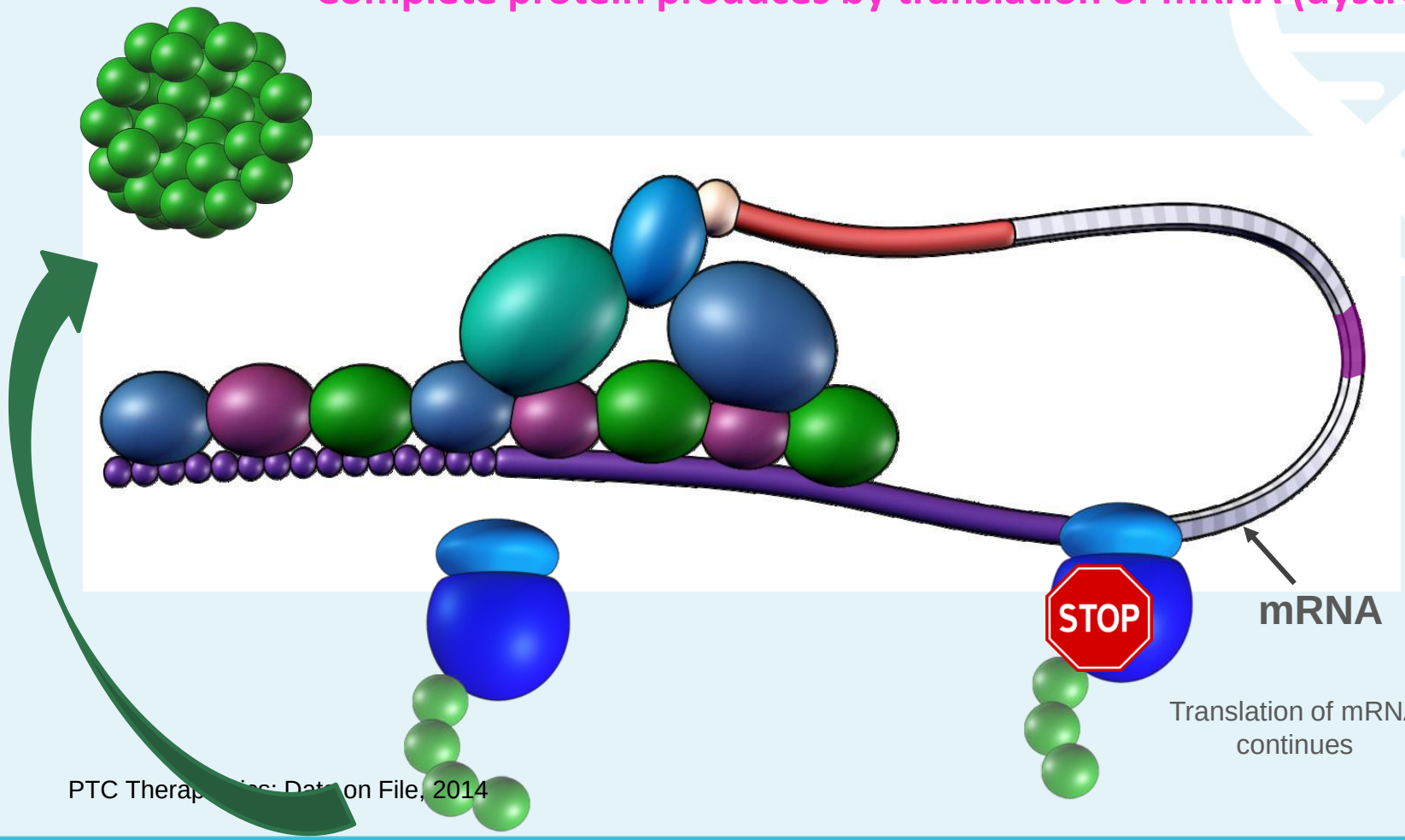
- Read through molecule is able to „overcome“ the premature STOP codon in mRNA
- Translation is going on up to end of mRNA
- Production of full-length functional protein

Normal STOP codon

PTC Therapeutics: Data on File, 2014

ATALUREN – read through molecule

Complete protein produces by translation of mRNA (dystrophin)

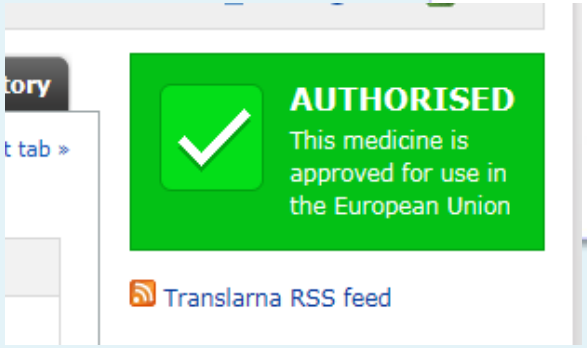


PTC Therap[illegible]s: Data on File, 2014

Which patient is a candidate for Ataluren/Translarna treatment?

PTC Therapeutics – TRANSLARNA (EMA, 2014 for nmDMD)

- DMD patient with non-sense mutation (nmDMD) confirmed by genetic testing
- Age: 5 years and older
- He/she is able to walk independently



~ 13% of DMD patients

European Medicines Agency: Translarna, EPAR, 2014,

www.ema.europa.eu, PTC Therapeutics: SmPC

Role of clinical geneticist

Clinical genetic examination and counseling + genetic tests

familiar anamnesis - genealogy

- information about occurence of:
  infertility, spontaneous miscarriages, stillbirths, affected children and adult members, mental retardation, cancer, consanguinity

- information about ethnical / geographic origin

- information about pregnancy
  (infectious diseases, metabolic disorders, use of alcohol, drugs ...)

physical and laboratory examination of individual

⇩ ⇩ ⇩ ⇩

DIAGNOSIS **PROGNOSIS** **PREVENTION** **THERAPY**

DIAGNOSIS

- diagnosis and type of inheritance, information about typical the course and development of disorder and variability of symptoms

PROGNOSIS

- calculation of risk of reccurence (general, Mendelian, empirical), detection and examination of other risk members of family, assessment of probability for disease expression

PREVENTION

- information about the reproductive options, primary prevention – preimplantation genetic diagnosis,
- secondary prevention – prenatal diagnosis
- postnatal examination of affected child

THERAPY

- treatment by specialist

OTHER INFORMATION AND ADVICE FOR PATIENT

- how to care about affected child, information about patient association, new medications and treatment options, psychological support

Principles of clinical genetic counseling

- To inform the patient and to give him information on the actual level of knowledge in medicine
- To explain all clearly on the level coresponding to intelect and education of the patient
- Non-directive counseling
- Discreetness
- Informed consent for genetic laboratory testing and to store genetic material (DNA)

Roles of a genetic register

- To maintain an informal two-way communication process between the family and the genetics unit
- To offer carrier detection to relevant family membbers as they reach adult life
- To coordinate presymptomatic and prenatal diagnosis when requested
- To coordinate multidisciplinary managment of patients with complex hereditary conditions such as familial cancer syndromes
- To ensure effective implementation of a new technology and treatment
- To provide a long-term source in information and support

reprogen
REPRODUKČNÁ GENETIKA

Thank you for your attention

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www.reprogen.sk