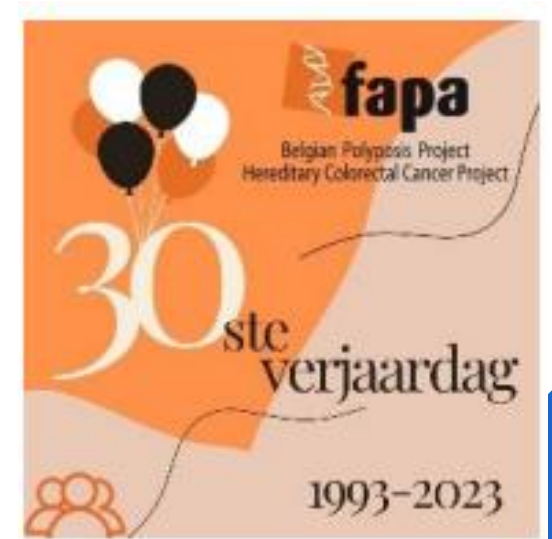
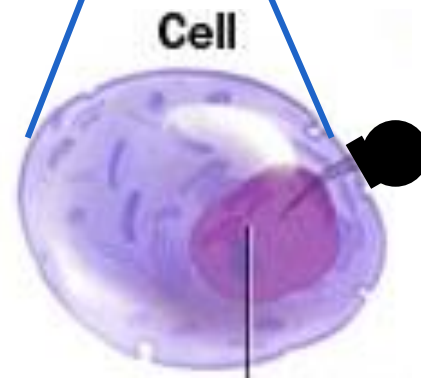


Evolutie van 30 jaar diagnostiek voor FAP en Lynch syndroom: evolutie in de genetische diagnostiek



De cel

The New Me in 80 Days
The race to replace the cells in my body!



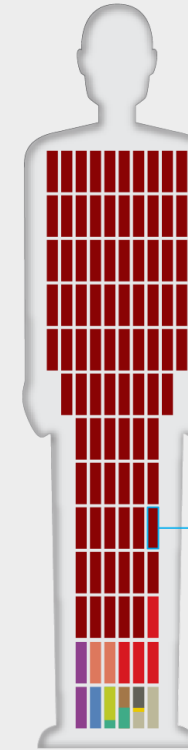
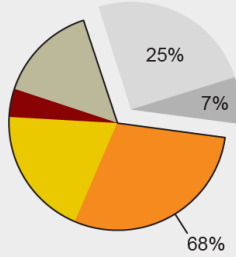
Nucleus = celkern

30 triljoen cellen ($30 \cdot 10^{12}$) in een menselijk lichaam

Cells in the Body

Number* A 70-kilogram male has roughly 30 trillion human cells. Fat and muscle cells are large—72 percent of cellular mass—but are only 0.1 percent of the total number. About 87 percent, by number, are erythrocytes—red blood cells—which are extremely small.

Mass About 25 percent of body mass is fluid outside of cells, such as plasma; another 7 percent is solids, such as minerals. That leaves 68 percent made of human cells.



Cell Type

Blood

- Erythrocytes
- Lymphocytes
- Neutrophils
- Monocytes

- Endothelial (vessels)
- Lung
- Hepatocytes (liver)
- Gastrointestinal lining
- Skin
- Brain
- Adipocytes (fat)
- Myocytes (muscle)
- Other

Little Cells Rule

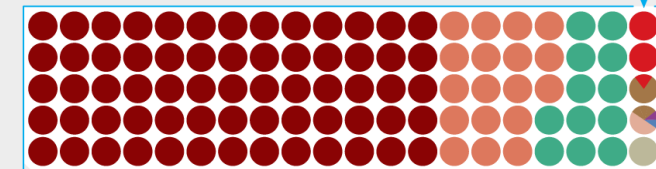
Large cells tend to live long, so daily turnover is dominated by plentiful, small cells with very short life spans.

- Erythrocytes**
Mass: 0.1 nanogram (ng)
Life span: 120 days
 - Colon epithelial cells**
1 ng
3–5 days
 - Muscle**
1,000–10,000 ng
30–70 years
- Many cells in the heart, eyes and brain last a lifetime.

Each rectangle represents 1 percent of all 30 trillion cells. That's roughly equivalent to the number of cells the body sheds and produces every day.

Cell Turnover per Day

By Number† Roughly 330 billion cells (+/-20 billion) turn over every day. About 86 percent are blood cells, and 12 percent are gut cells. Other cells are replaced very slowly.



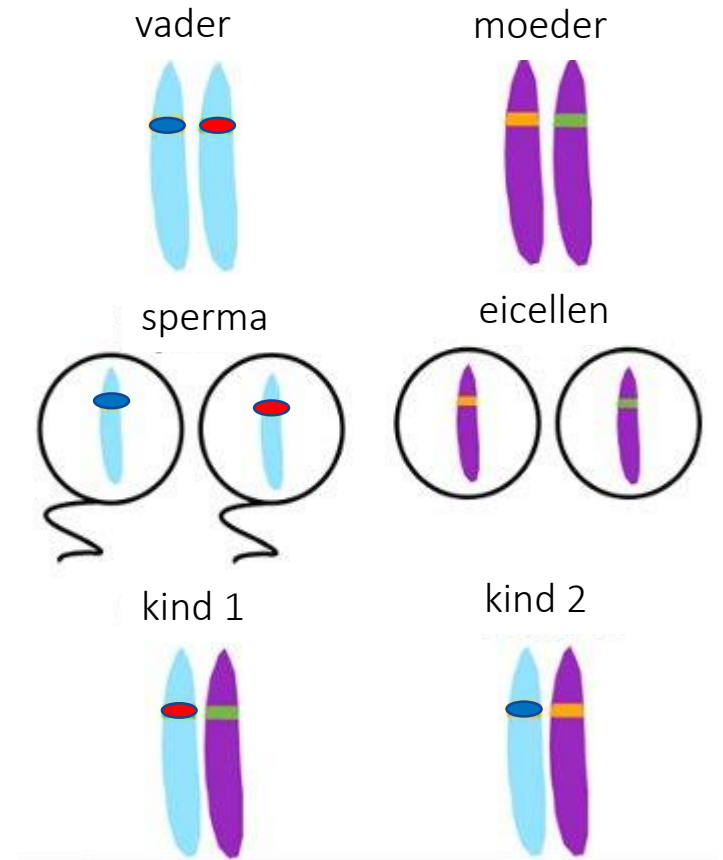
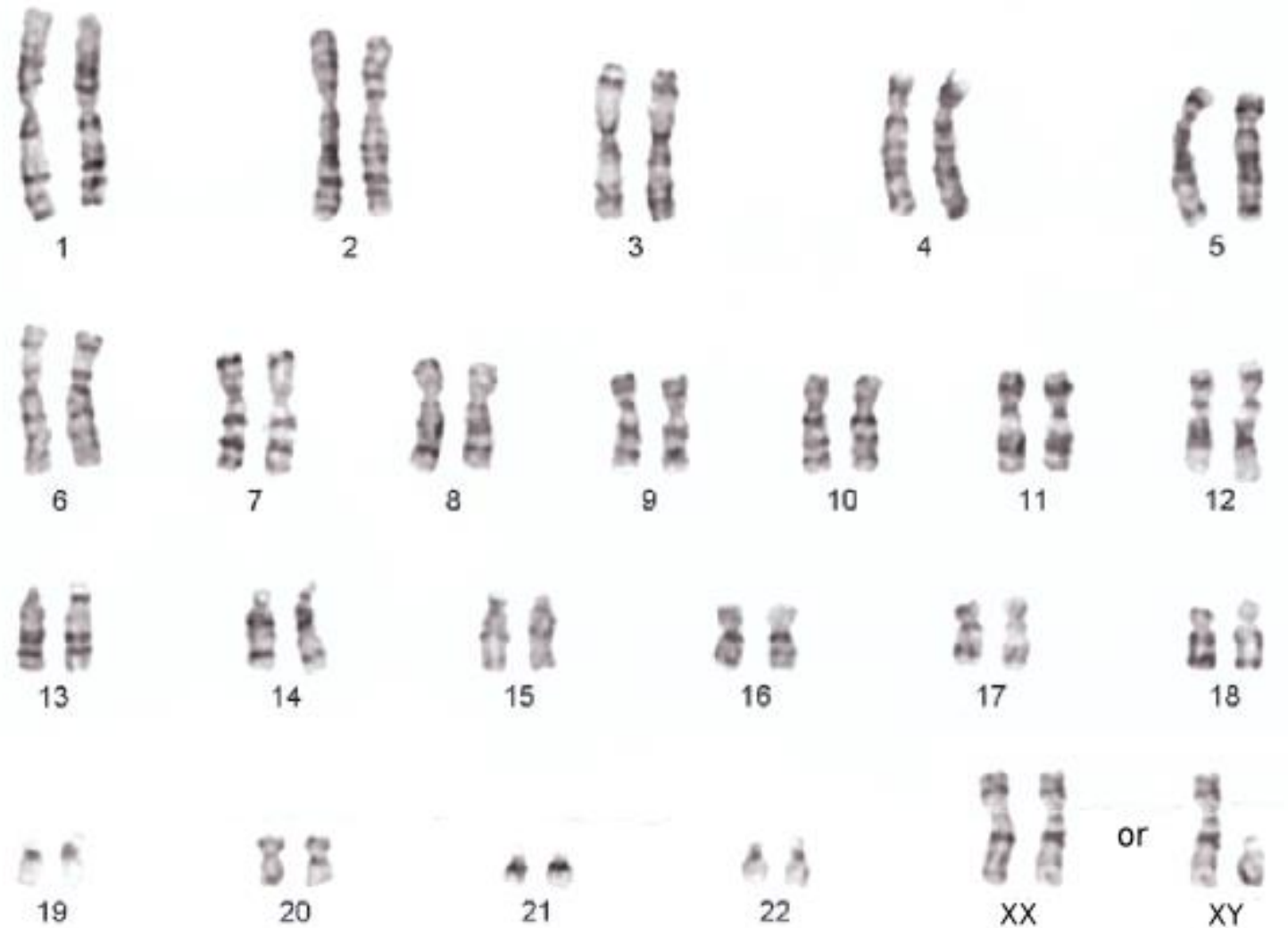
By Mass About 49 percent are blood cells, 41 percent are gut cells, with skin making up 4 percent, fat 4 percent and muscle 1 percent. Daily mass turnover is 80 grams (+/-20).



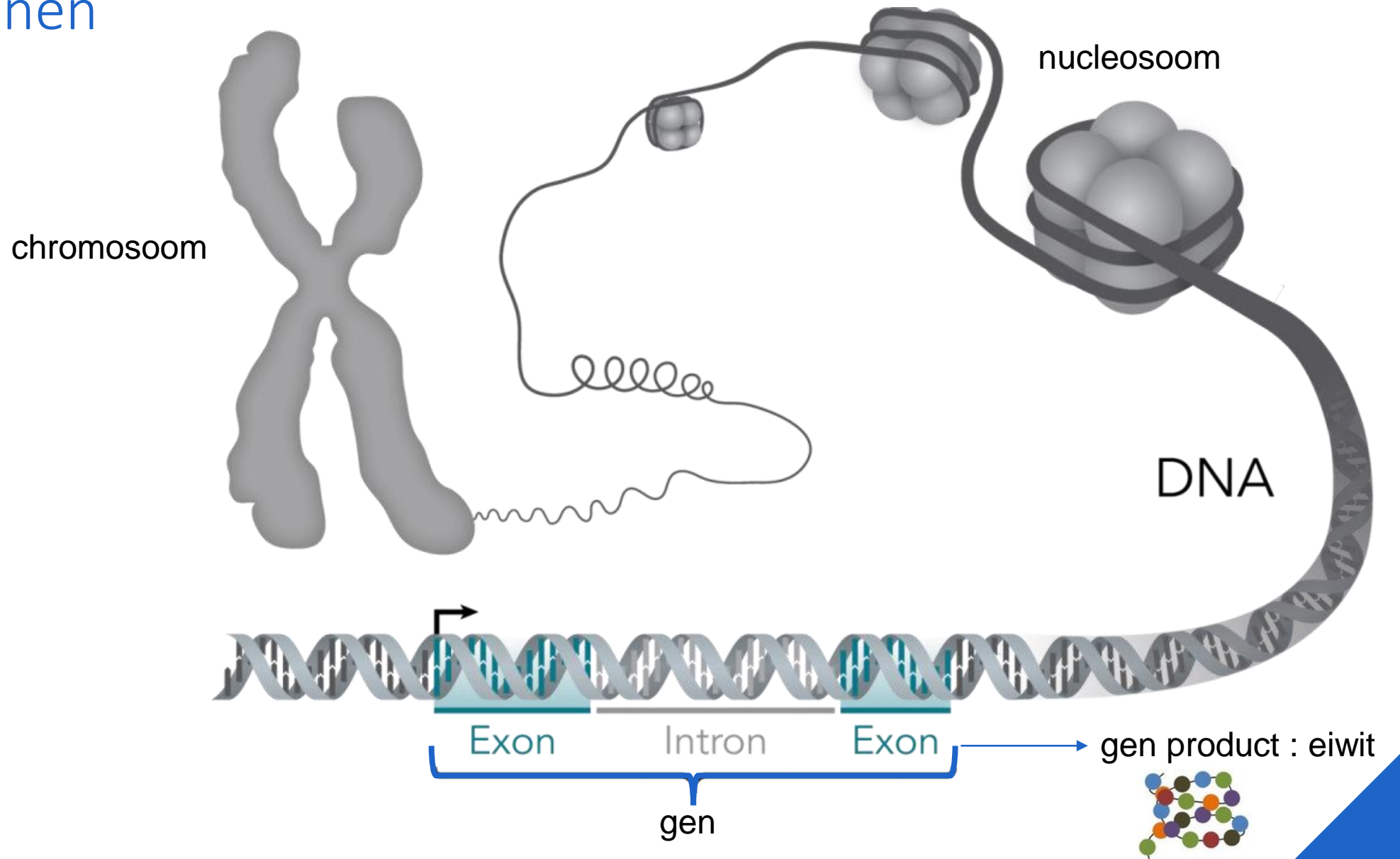
**This research is rooted in a standard reference person, which historically has been defined as a male, age 20 to 30, weighing 70 kilograms. Cells lost or gained resulting from menstruation were not taken into account. Negligible percentages are not shown.*

†Our bodies harbor another 38 trillion bacteria and many more viruses, but they weigh only 200 to 300 grams (seven to 11 ounces) and are not counted as human.

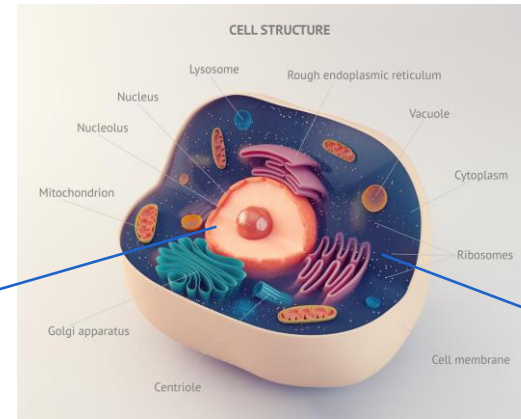
Het humane genoom: 23 paar chromosomen



Onze genen



Het centrale dogma van de moleculaire biologie

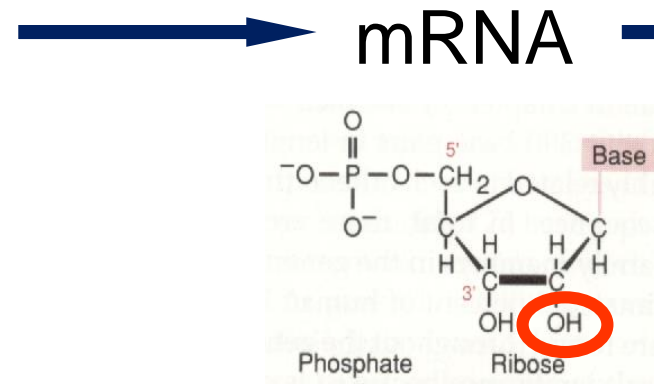
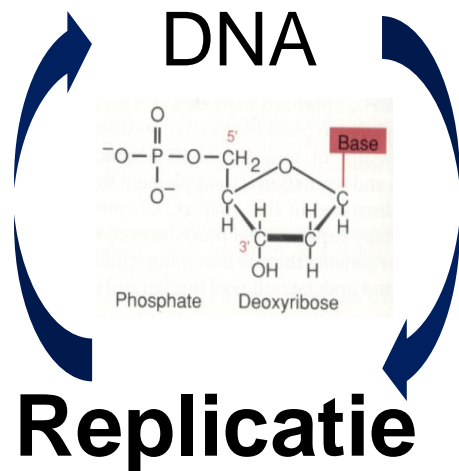
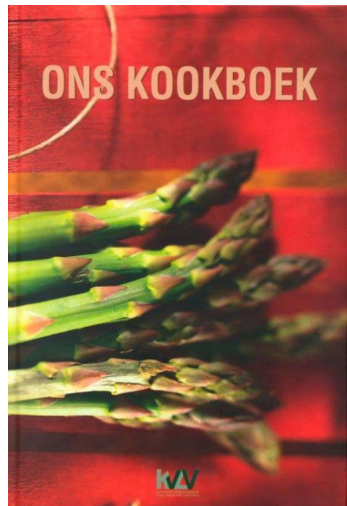


celkern

cytoplasma

Transcriptie

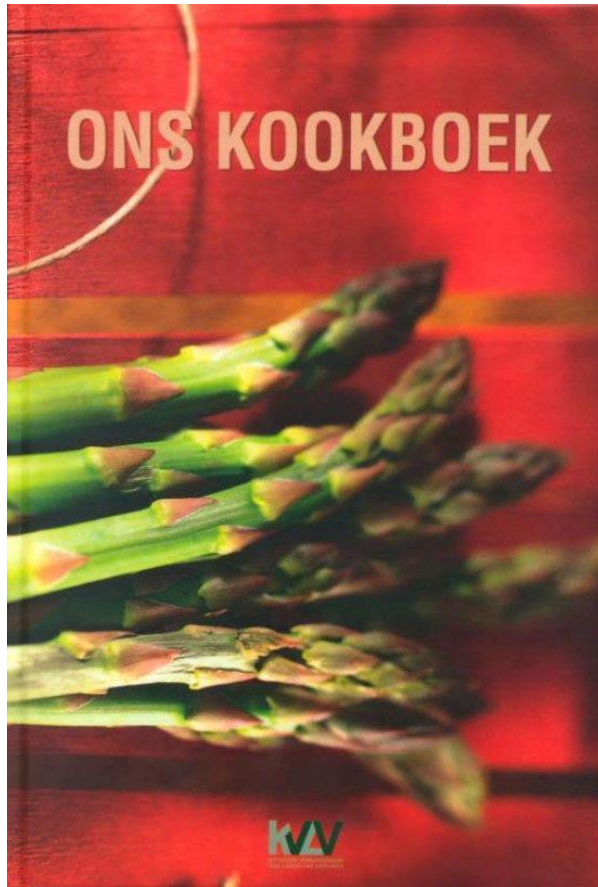
Translatie



Eiwit



Veranderingen in het genoom



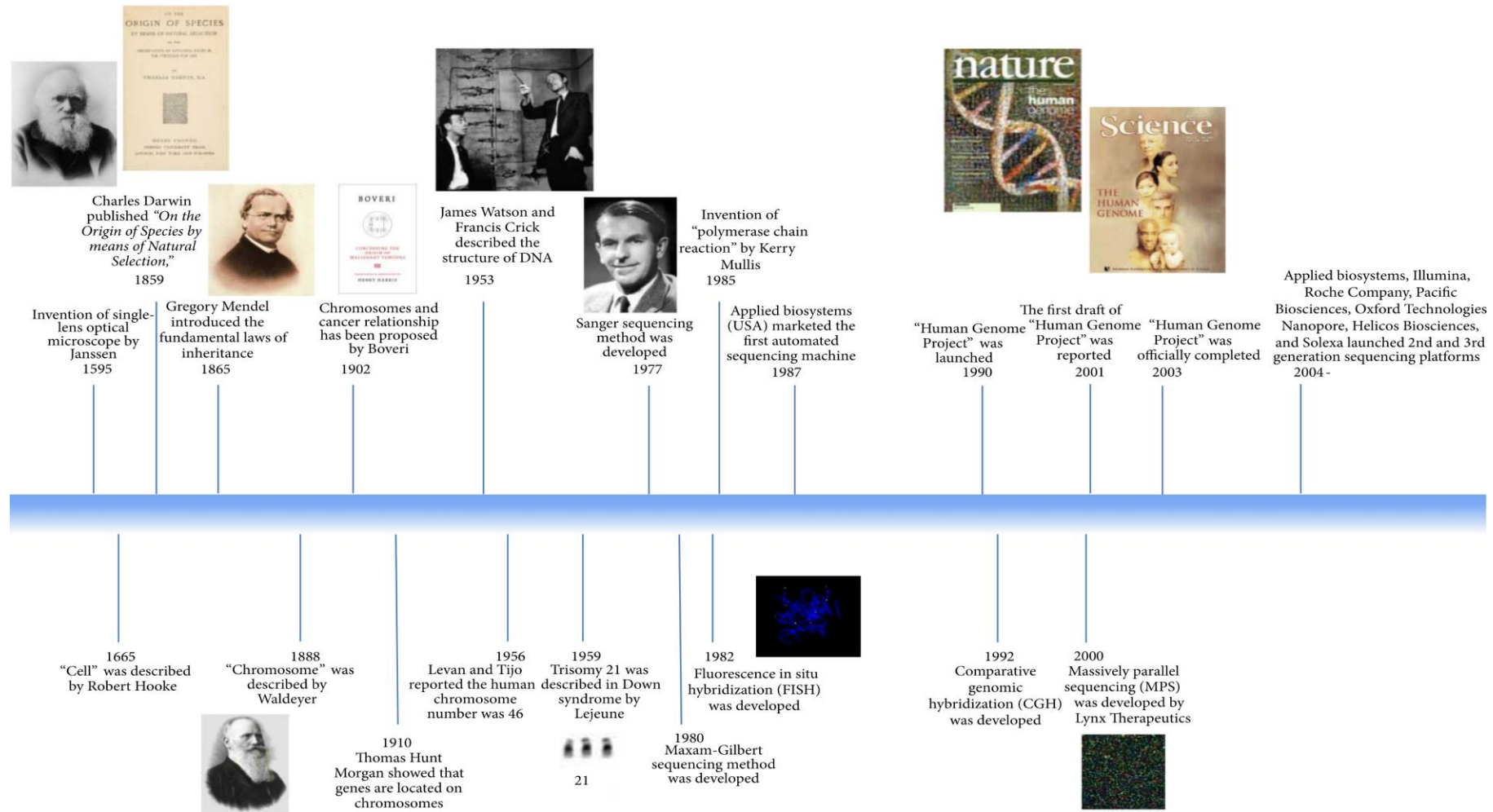
“...maak de bereiding van het product lichtjes zouter door...”

“...maak de bereiding van het produkt lichtjes zouter door...”

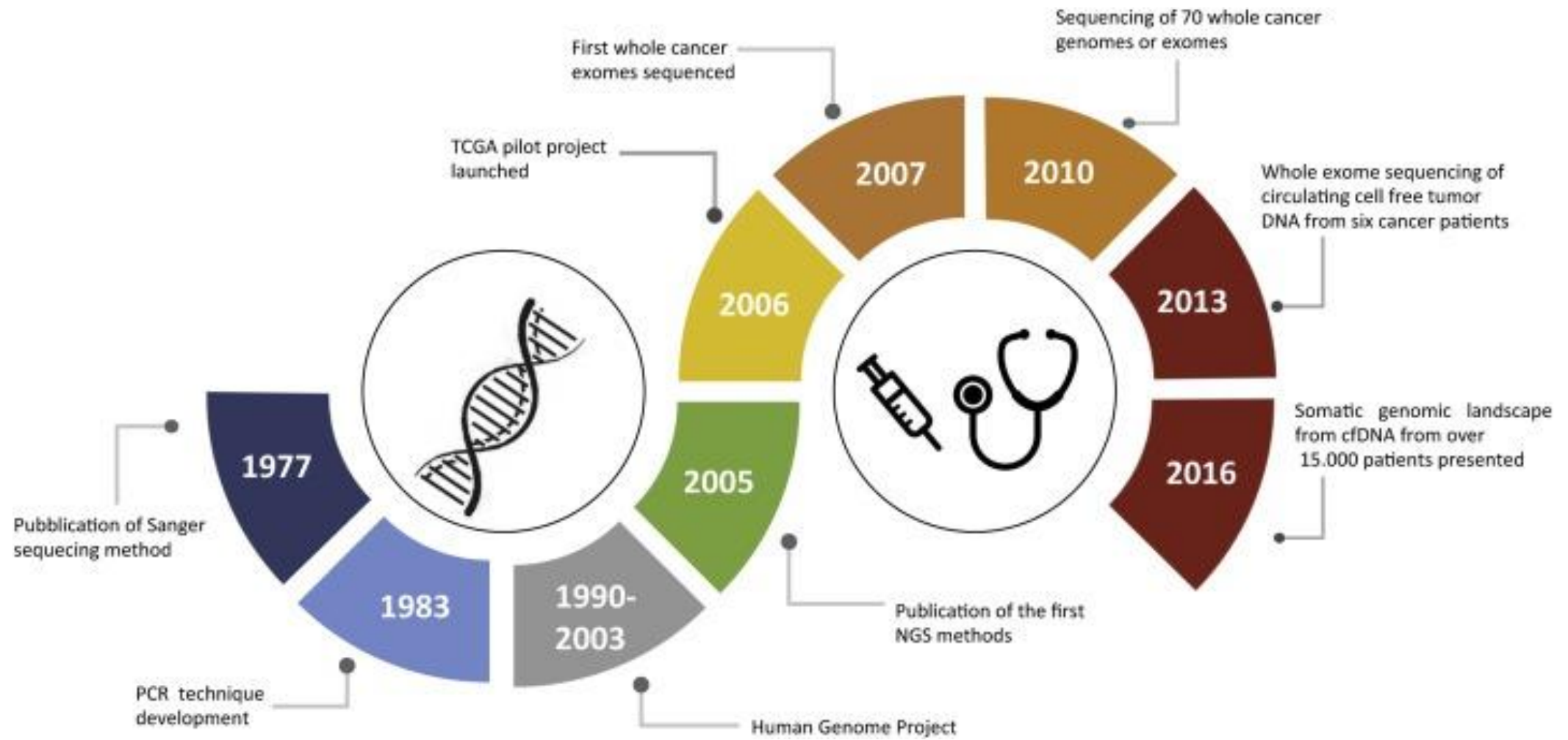
“...maak de berijding van het product lichtjes zouter door...”

“...maak de bereiding van het product lichtjes zoeter door...”

Historiek genetische technieken



Mijlpalen...



Het humane genoom



4 bouwstenen: A, C, G, T
3 miljard letters





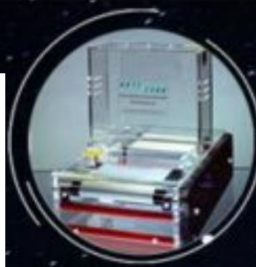
1869
Friedrich Miescher isolates DNA, in the form of chromatin



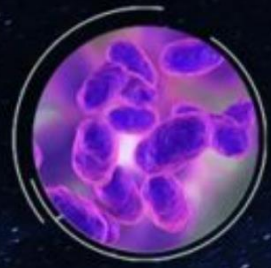
1965
Robert Holley and colleagues sequence yeast tRNA



1975
Frederick Sanger introduces "PLUS AND MINUS" method for DNA sequencing using gel electrophoresis to separate newly synthesized DNA by size



1984
Fritz Pohl develops NON-RADIOACTIVE sequencing platform



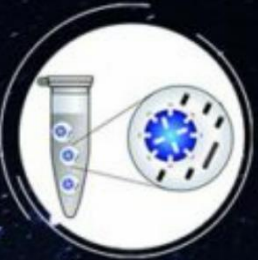
1995
Fun and Facts Science News
genome sequenced:
H. INFLUENZAE



1998
Solexa develops "sequencing-by-synthesis" method that uses fluorescent dyes
C. ELEGANS genome is sequenced



2002
Complete MOUSE genome is sequenced



2007
SOLiD system, which uses SEQUENCING BY LIGATION, is launched



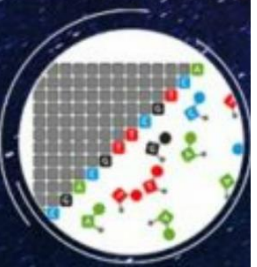
2011
Pacific Biosciences launches first commercial product - PacBio RS - based on SINGLE MOLECULE REAL TIME technology

1953
James Watson and Francis Crick discovered the structure of DNA as a DOUBLE HELIX



1972
Walter Fiers sequences first whole gene: coding for a MS2 virus protein

1977
Frederick Sanger establishes the CHAIN TERMINATION METHOD and uses it to sequence first complete genome: bacteriophage ϕ X174



1987
Leroy Hood and ABL develop first semi-automated DNA sequencing machines



1996
Ronaghi, Uhlen and Nyren introduce PYROSEQUENCING, a "sequencing-by-synthesis" method

ABI introduces first commercial sequencing using capillary electrophoresis

S. CEREVISIAE genome is sequenced

2. November 2020

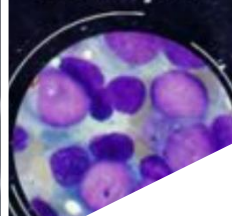
1999
First human chromosome 22 is sequenced



2005
The 454 SYSTEM, based on pyrosequencing, becomes the first next-generation sequencer to come on the market



2008
Whole DNA sequence of a CANCER is decoded for the first time

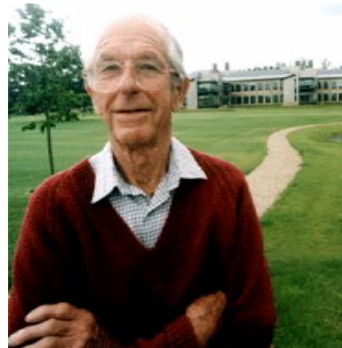
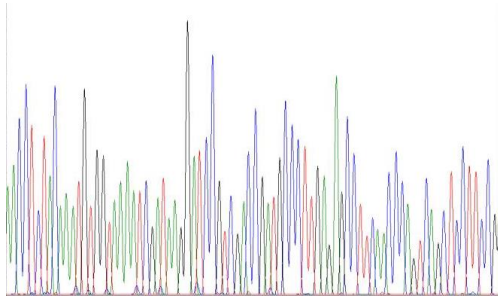


2012
Oxford Nanopore Technologies commercializes NANOPORE-SEQUENCING



A Journey Through The History Of DNA Sequencing

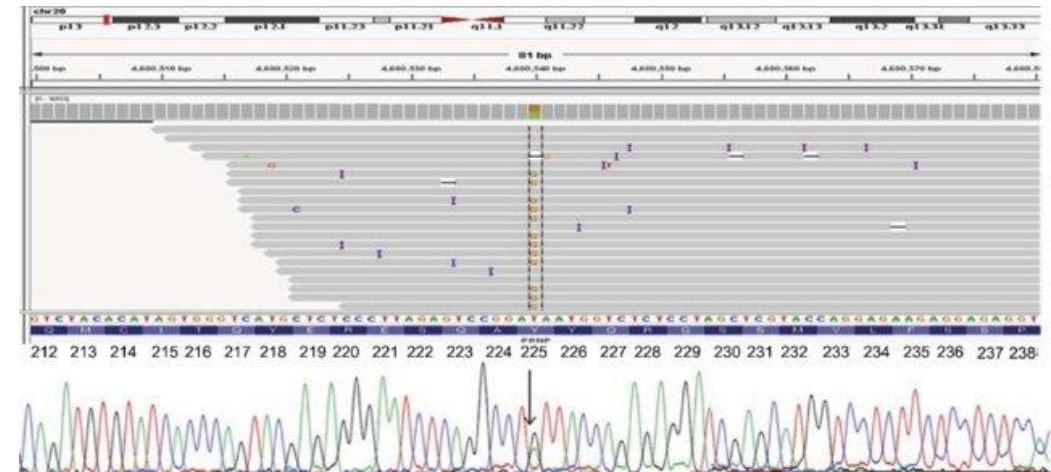
Sequencing – grote evolutie



Fred Sanger Nobel
price 1980

1 gen
(20 exonen: 1 maand voor 8 patiënten)
800 euro

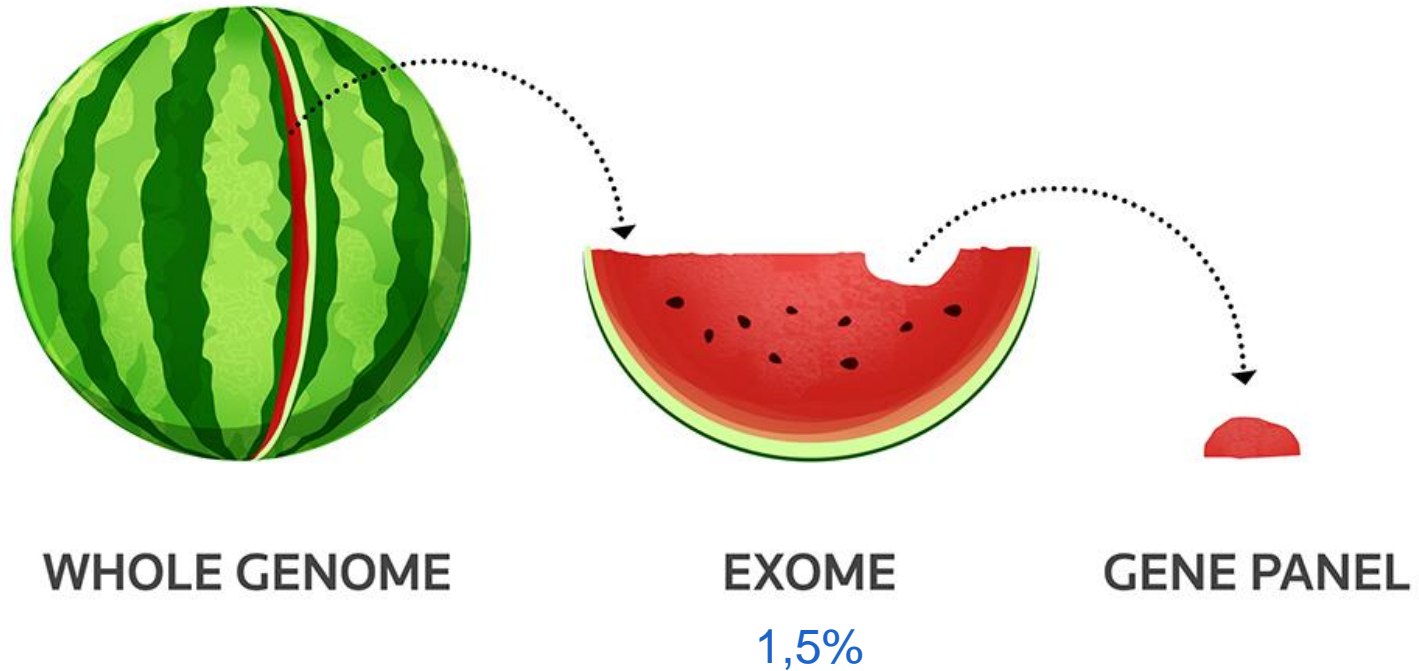
Sanger Sequencing



20 000 genen
250 Euro
'1 week'

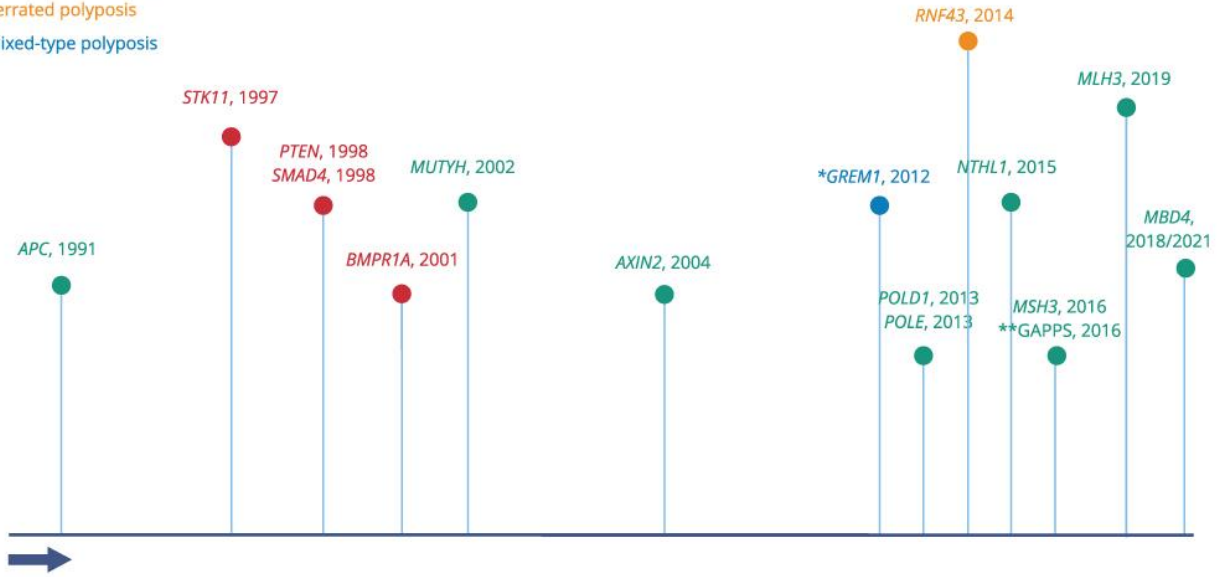
Next-generation sequencing

Genetische diagnostiek: types van genetische testen



polyposis

Adenomatous polyposis
 Hamartomatous polyposis
 Serrated polyposis
 Mixed-type polyposis



1990
 The Human Genome Project begins
 Aim: to sequence the human genome

1996
 'Bermuda principles'
 Researchers agree that the human genome data will be made publicly available to advance healthcare research worldwide

2001
 Publication of a 'rough draft' of the human genome, comprising 90% of the total

2003
 The Human Genome project is completed

2005
 Next-generation sequencing (NGS) technologies become commercially available

2012/2013
 CRISPR-Cas9 is successfully used for genome editing of eukaryotic cells

HISTOLOGY	GENE (CLINICAL ENTITY)	INHERITANCE	PATHWAY
Adenomatous	APC (familial adenomatous polyposis, FAP)* AXIN2 (oligodentia-colorectal cancer syndrome) POLD1 (polymerase proofreading-associated polyposis, PPAP) POLE (polymerase proofreading-associated polyposis, PPAP)	Autosomal dominant	WNT/ β -catenin signaling pathway WNT/ β -catenin signaling pathway DNA proofreading DNA proofreading
Adenomatous	MBD4 (MBD4-associated neoplasia syndrome, MNS) MLH1, MSH2/6, PMS2 (constitutional mismatch repair syndrome, CMMRD) MLH3 (MLH3-related polyposis) MSH3 (MSH3-related polyposis) MUTYH (MUTYH-associated polyposis, MAP) NTHL1 (NTHL1 tumor syndrome)	Autosomal recessive	Base excision repair pathway MMR pathway MMR pathway MMR pathway Base excision repair pathway Base excision repair pathway
Hamartomatous	BMPR1A (juvenile polyposis syndrome) PTEN (PTEN hamartoma tumor syndrome) SMAD4 (juvenile polyposis syndrome) STK11 (Peutz-Jeghers syndrome)	Autosomal dominant	TGF- β /BMP signaling pathway PTEN-AKT-PI3K pathway TGF- β /BMP signaling pathway mTOR signaling pathway
Serrated	RNF43 (serrated polyposis syndrome)	Autosomal dominant	WNT/ β -catenin signaling pathway
Mixed-type	GREM1 (hereditary mixed polyposis syndrome)**	Autosomal dominant	TGF- β /BMP signaling pathway

nonpolyposis

MMR genes	Chromosome Location	Frequency
MSH2	2p16	45-50%
MLH1	3p22	20%
MSH6	2p16	10%
PMS2	7p22	1%
PMS1	2q32	Rare
MSH3	5q14.1	Rare
EXO1	1q43	Rare
Other unknown genes		20-25%

EPCAM

2p16

<1%

The Clinical Spectrum of EPCAM Mutations

2 wild type EPCAM alleles

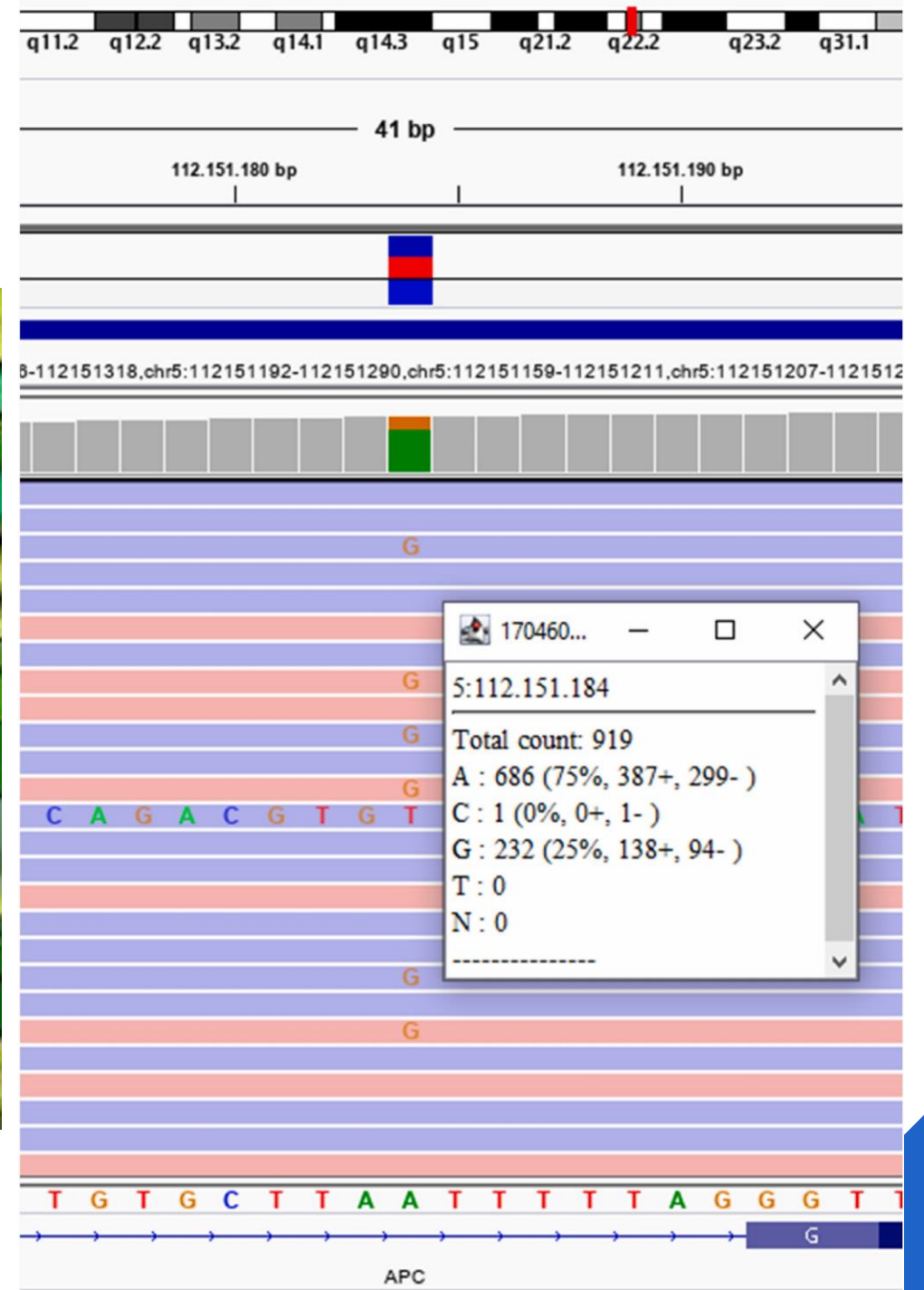
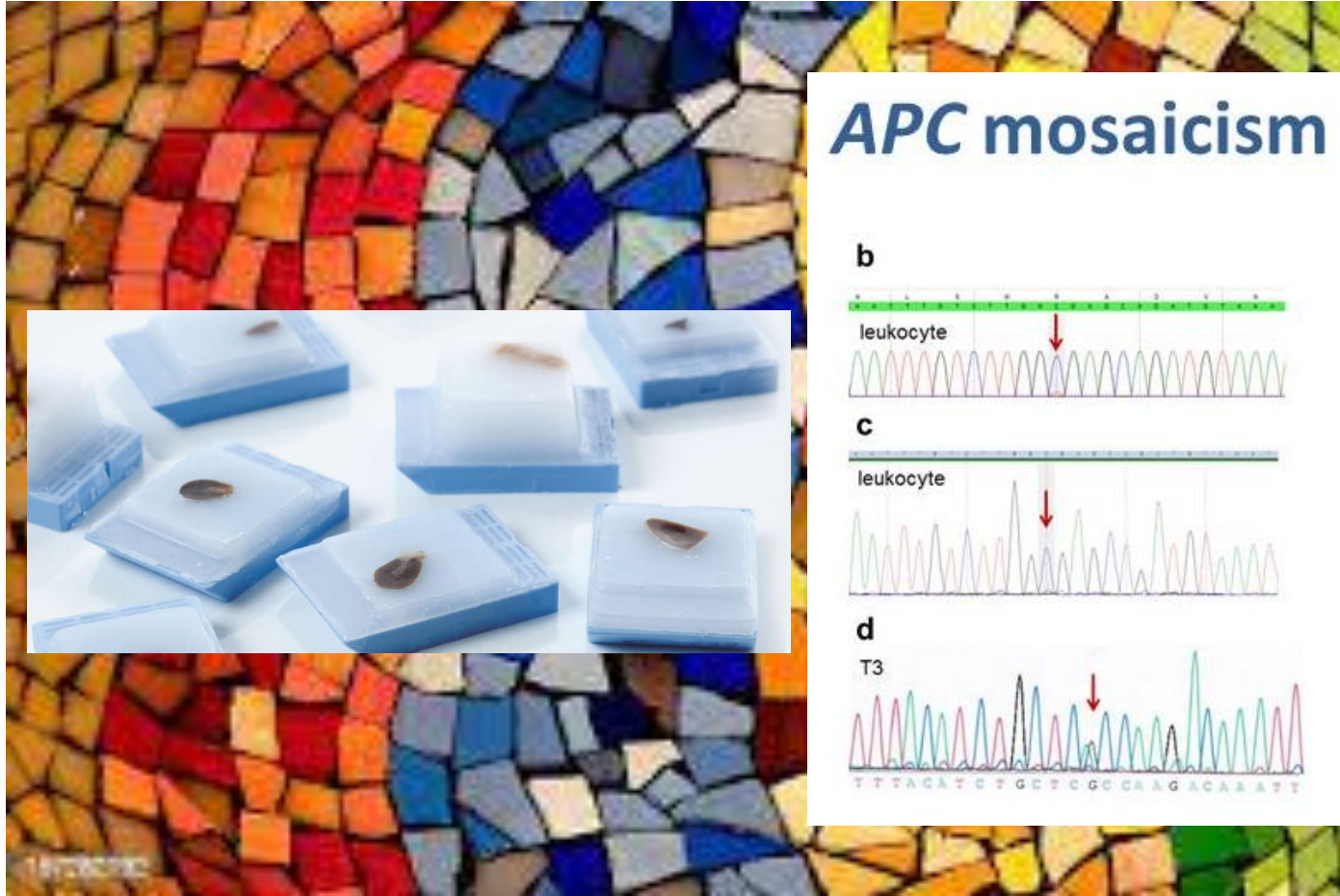
 Normal Phenotype

One wild type EPCAM allele,
 one with a partial deletion

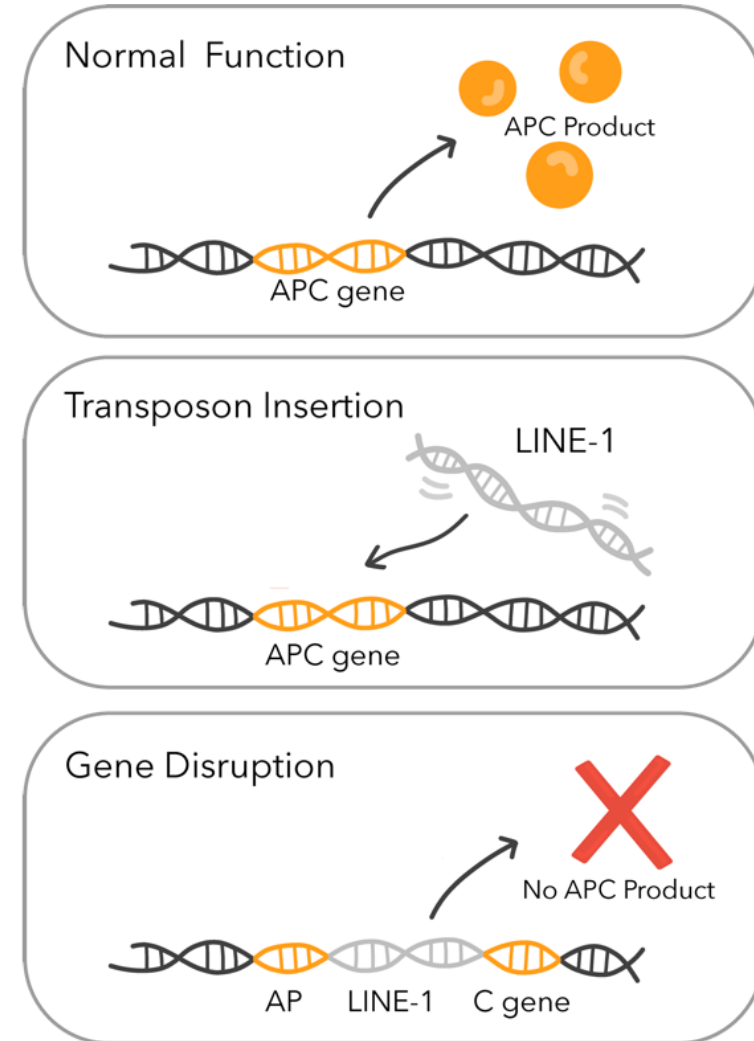
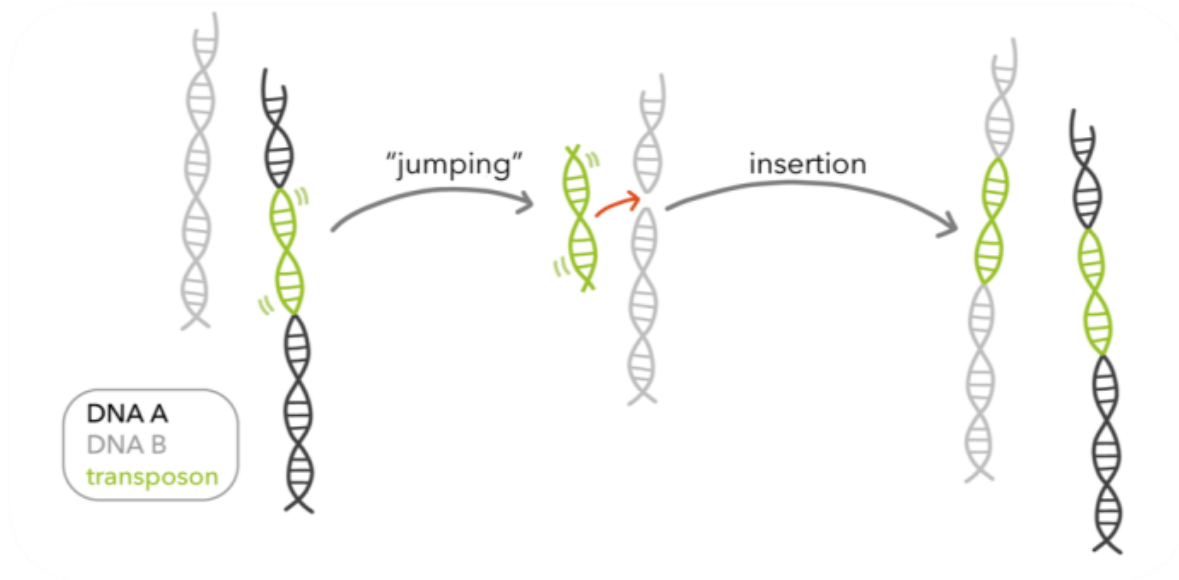
 Lynch Syndrome

Missing genetic variations...

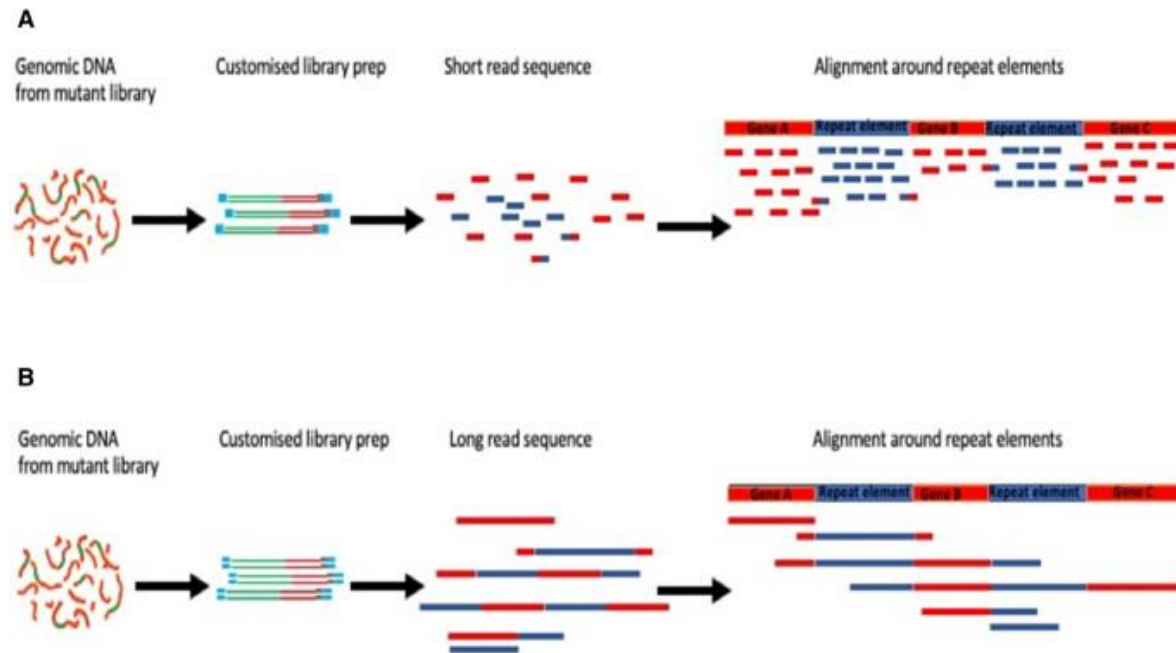
Mozaïeken



“jumping” genes: transposons



Transposons detecteren via long read sequencing



[Insertion of an SVA element in MSH2 as a novel cause of **Lynch** syndrome.](#)

3 Yang C, Li Y, Trottier M, Farrell MP, Rai VK, E Salo-Mullen E, Gallagher DJ, Stadler ZK, van der Klift HM, Zhang L.

Cite Genes Chromosomes Cancer. 2021 Aug;60(8):571-576. doi: 10.1002/gcc.22950. Epub 2021 Apr 21.
Share PMID: 33822432

Germline mutations in the DNA mismatch repair (MMR) genes cause **Lynch** syndrome (LS). In this study, we identified and characterized a novel SINE-VNTR-Alu (SVA) insertion in exon 12 of MSH2 in an individual with early-onset colorectal cancer and a very strong LS family hist ...

[SVA retrotransposon insertion in exon of MMR genes results in aberrant RNA splicing and causes **Lynch** syndrome.](#)

4 Yamamoto G, Miyabe I, Tanaka K, Kakuta M, Watanabe M, Kawakami S, Ishida H, Akagi K.

Cite Eur J Hum Genet. 2021 Apr;29(4):680-686. doi: 10.1038/s41431-020-00779-5. Epub 2020 Dec 8.
Share PMID: 33293698 [Free PMC article.](#)

Lynch syndrome is an autosomal dominant hereditary cancer syndrome in which many cancers develop, the main one being colorectal cancer. ...The insertion **sequences** were ~2.5 kbp in length and were found to have the structure of an SVA retrotransposon (SVA). ...

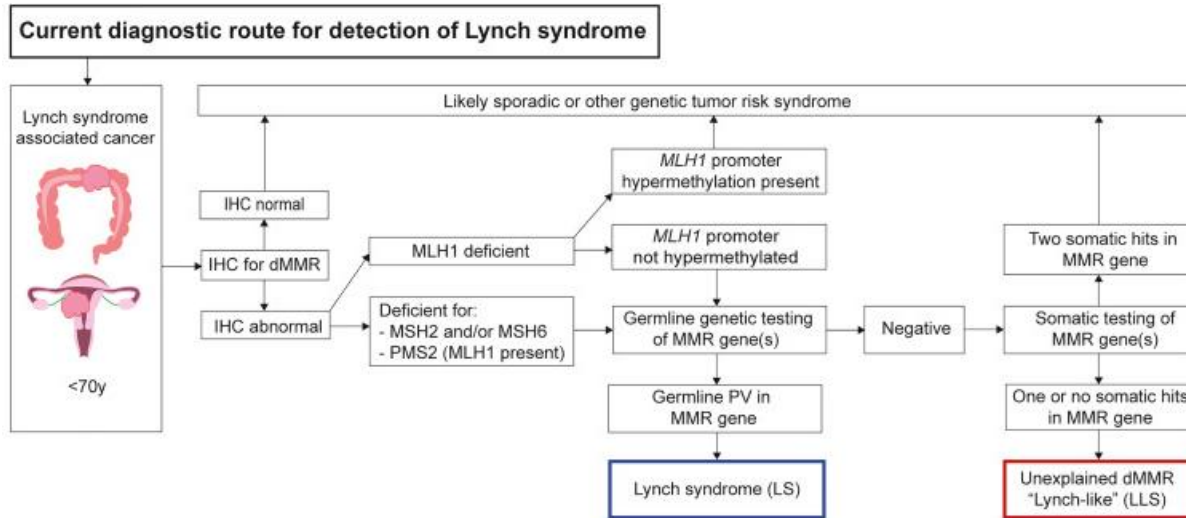
[Insertion of an Alu-like element in MLH1 intron 7 as a novel cause of **Lynch** syndrome.](#)

5 Li Y, Salo-Mullen E, Varghese A, Trottier M, Stadler ZK, Zhang L.

Cite Mol Genet Genomic Med. 2020 Dec;8(12):e1523. doi: 10.1002/mgg3.1523. Epub 2020 Oct 15.
Share PMID: 33058565 [Free PMC article.](#)

BACKGROUND: **Lynch** Syndrome (LS) is caused by germline mutations in the DNA mismatch repair (MMR) genes with mutations in MLH1 accounting for ~40% of LS-related alterations. ...Immunohistochemistry for MMR genes and MLH1 promoter methylation were analyzed on patient's ...

Varianten buiten de coderende regio's



Constitutional chromothripsis of the *APC* locus as a cause of genetic predisposition to colon cancer

Florentine Scharf¹, Rafaela Magalhaes Leal Silva¹, Monika Morak¹, Alex Hastie², Julia M A Pickl¹, Kai Sendelbach¹, Christian Gebhard¹, Melanie Locher¹, Andreas Laner¹, Verena Steinke-Lange¹, Udo Koehler¹, Elke Holinski-Feder^{1, 3}, Dieter A Wolf^{1, 4}

Correspondence to Dr Dieter A Wolf, Medizinisch Genetisches Zentrum, Munchen 80335, Bayern, Germany; dieter.wolf@gmgz-muenchen.de; Professor Elke Holinski-Feder; elke.holinski-feder@gmgz-muenchen.de

Abstract

Purpose Approximately 20% of patients with clinical familial adenomatous polyposis (FAP) remain unsolved after molecular genetic analysis of the *APC* and other polyposis genes, suggesting additional pathomechanisms.

Methods We applied multidimensional genomic analysis employing chromosomal microarray profiling, optical mapping, long-read genome and RNA sequencing combined with FISH and standard PCR of genomic and complementary DNA to decode a patient with an attenuated FAP that had remained unsolved by Sanger sequencing and multigene panel next-generation sequencing for years.

Results We identified a complex 3.9 Mb rearrangement involving 14 fragments from chromosome 5q22.1q22.3 of which three were lost, 1 reinserted into chromosome 5 and 10 inserted into chromosome 10q21.3 in a seemingly random order and orientation thus fulfilling the major criteria of chromothripsis. The rearrangement separates *APC* promoter 1B from the coding ORF (open reading frame) thus leading to allele-specific downregulation of *APC* mRNA. The rearrangement also involves three additional genes implicated in the *APC*-Axin-GSK3B-β-catenin signalling pathway.

Conclusions Based on comprehensive genomic analysis, we propose that constitutional chromothripsis dampening *APC* expression, possibly modified by additional *APC*-Axin-GSK3B-β-catenin pathway disruptions, underlies the patient's clinical phenotype. The combinatorial approach we deployed provides a powerful tool set for deciphering unsolved familial polyposis and potentially other tumour syndromes and monogenic diseases.



Gastroenterology aga

RESEARCH LETTER | VOLUME 163, ISSUE 6, P1691-1694.E7, DECEMBER 2022

Noncoding Aberrations in Mismatch Repair Genes Underlie a Substantial Part of the Missing Heritability in Lynch Syndrome

Iris B.A.W. Te Paske • Arjen R. Mensenkamp • Kornelia Neveling • ERN-GENTURIS Lynch-Like Working Group • Nicoline Hoogerbrugge • Marjolijn J.L. Ligtenberg • Richarda M. De Voer • Show less

Open Access • Published: August 26, 2022 • DOI: <https://doi.org/10.1053/j.gastro.2022.08.041>

Variant classificatie



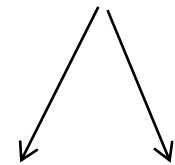
Germline variants: 5 classes

- **Class 5**
 - Predicted to be **pathogenic**, this result therefore **confirms** the diagnosis
- **Class 4**
 - **Likely** pathogenic, **consistent** with the diagnosis
- **Class 3**
 - **Uncertain** pathogenicity, **does not confirm or exclude** diagnosis
 - Unsure about the pathogenicity and offer further work before offering further diagnostic or carrier testing.
- **Class 2**
 - **Unlikely** to be pathogenic, diagnosis not confirmed molecularly.
 - No evidence suggesting pathogenicity but not at a high enough frequency to say it's not pathogenic?
- **Class 1**
 - **Not** pathogenic.
 - “Common” polymorphism.
 - No evidence suggesting pathogenicity and at “high” frequency.

Predictieve test
Prenatale test
PGT

pre-implantatie genetische diagnostiek

- vermijden van ernstige aandoeningen zonder nood aan een zwangerschapsafbreking
- IVF procedure met nazicht van het genetisch defect op de bekomen embryo's en terugplaatsen van embryo's zonder het genetisch defect
 - ▶ Gendefect moet gekend zijn
 - ▶ Oppuntstelling nodig
 - ▶ Langdurig traject
 - ▶ Psychologisch belastend (terugplaatsing: 30% zwangerschapskans)
 - ▶ Lichamelijk belastend voor de vrouw
 - ▶ Grotendeels terugbetaald (6 stimulaties - <42 jaar)



ACGGTCCTTTGAAG
ACGGTCCTTTGAAG



ACGGTCCTTTGAAG
ACGGTC---TGAAG



Genetisch onderzoek - waar kan je terecht?

- erfelijkheidsonderzoek werpt vele vragen op
- voor deskundig advies kun je terecht in een genetisch centrum
- genetische centra zijn verbonden aan een universitair ziekenhuis
- Alle genetische centra hebben samenwerkingsovereenkomsten met algemene ziekenhuizen (78)

UZ Gent

1. UZ Gent
2. AZ St-Jan Brugge
3. AZ Delta Roeselare
4. AZ Alma Eeklo
5. AZ Maria-Middelares Gent
6. AZ Jan Palfijn Gent
7. AZ St-Lucas Gent
8. AZ Zorgsaam Terneuzen
9. AZ Nikolaas St-Niklaas
10. AZ St-Elisabeth Zottegem
11. MPI 't Veld Aartrijke Zeldegem

UZ Leuven

1. UZ Leuven Gasthuisberg
2. OLV Ziekenhuis, Aalst
3. AZ Imelda, Bonheiden
4. AZ Sint-Jan, Brugge
5. AZ Sint-Basilius, Dendermonde
6. ZOL André Dumont, Genk
7. Jessa Ziekenhuis, Hasselt
8. Jan Yperman Ziekenhuis, leper
9. AZ Groeninge, Kortrijk
10. AZ Sint-Maarten, Mechelen
11. AZ Damiaan, Oostende
12. AZ Turnhout, Turnhout
13. GZA Ziekenhuizen, Wilrijk
14. AZ Sint Lucas, Brugge

UZ Antwerpen

1. UZ Antwerpen
2. AZ Klina, Brasschaat
3. GZA, Wilrijk
4. AZ Voorkempen, Malle
5. AZ Rivierenland, Bornem
6. Aalst Stedelijk Ziekenhuis, Aalst
7. AZ Dimpna, Geel
8. Heilig Hart AZ, Lier
9. ZNA Middelheim ziekenhuis, Antwerpen
10. ZNA Paola ziekenhuis, Antwerpen
11. Radboud Universiteit Medical Center, Nijmegen

UZ Brussel

1. UZ Brussel
2. ZNA Middelheim
3. CHU Brugmann
4. ASZ Aalst
5. OLV Campus Asse
6. Polikliniek Dilbeek

UZL

1. CUSL, Woluwe
2. Clinique Saint Jean, Brussels
3. Clinique Saint Pierre Ottignies
4. Clinique Louvain la neuve
5. Clinique CHR Mouscron
6. William Lennox, Ottignies

IPG Gosselies

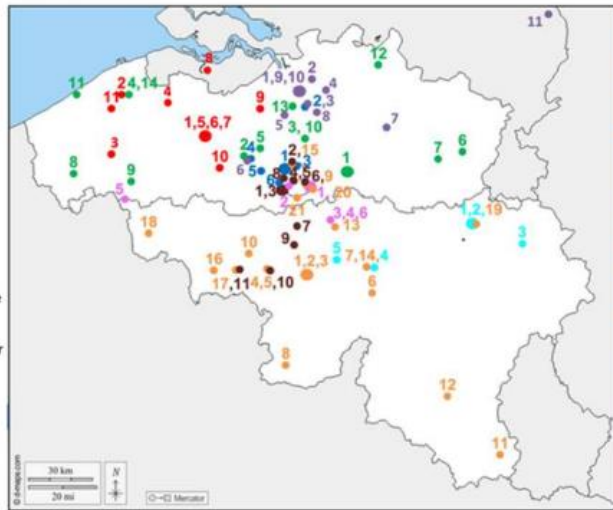
1. IPG Gosselies
2. GhDC Charleroi
3. ISPPC Charleroi
4. Hôp. Jolimont, La Louvière
5. CHUT Tivoli, La Louvière
6. CHU UCL Mont-Godinne
7. CHU UCL Namur
8. CSF Chimay
9. CHIREC Delta, Brussels
10. CHR Haute Seine, Soignies
11. VIVALIA CLS Arlon
12. VIVALIA, Libramont
13. La Petite Maison, Chastres
14. Les Perce-Neige, Jambes
15. HUDERF, Brussels
16. EPICURA site Hornu, Hornu
17. CHU Ambroise-Paré, Mons
18. CHWAPI, Tournai
19. CHC MontLegia, Liège
20. CUSL, Woluwe
21. IRSA, Uccle

ULB

1. Hôpital Erasme
2. HUDERF Children Hospital
3. Institut Jules Bordet
4. CHU St Pierre, Brussels
5. Hôpital Etterbeek-Ixelles
6. CHIREC Delta, Brussels
7. CHIREC Braine l'Alleud
8. Clinique Ste Anne St Rémi
9. Centre Méd. J.Monnet, Nivelles
10. Hôpital Tivoli La Louvière
11. CHU Ambroise-Paré, Mons

ULiège

1. CHC MontLegia, Liège
2. CHR La Citadelle Liège
3. CHR Verviers
4. CHR Namur
5. CHRVS Auvclais



<https://www.uzgent.be/patient/zoek-een-arts-of-dienst/centrum-voor-medische-genetica>

Informatie – waar kan je terecht?



For patients

European Patient Advocacy Groups

Patient organisations can become member organisations of the ERN GENTURIS European Patient Advocacy Group (ePAG).
[Read more >>](#)

Patient associations

A non-exhaustive list of patient associations for genetic tumour risk syndromes in EU member states can be found [here](#).

Patient Journeys

ERN GENTURIS Members and patient representatives have developed an innovative visual approach for the clinical pathway of patients with rare diseases: "Patient Journeys".
[Read more >>](#)

Find a HCP

The current list of participating healthcare providers covers 22 EU Member States and Norway.
[Read more >>](#)



Share. Care. Cure.

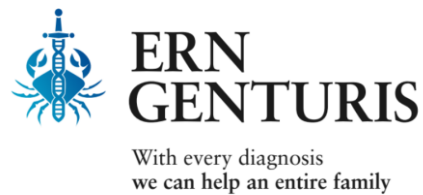


**ERN
GENTURIS**

With every diagnosis
we can help an entire family



Vragen?



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