Prof dr ir Kathleen Claes / Centrum voor medische genetica

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

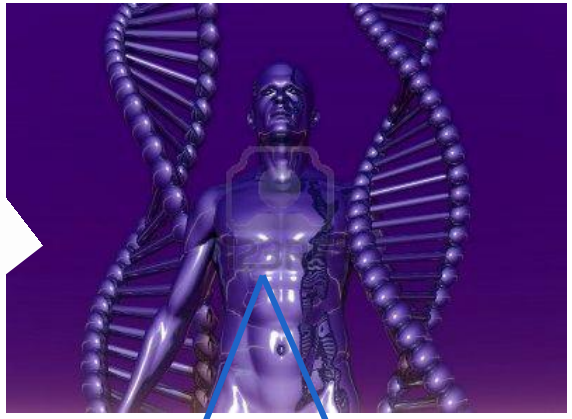
UZ GENT
UNIVERSITEIT GENT
CRIG
ERN GENTURIS
With every diagnosis
we can help an entire family

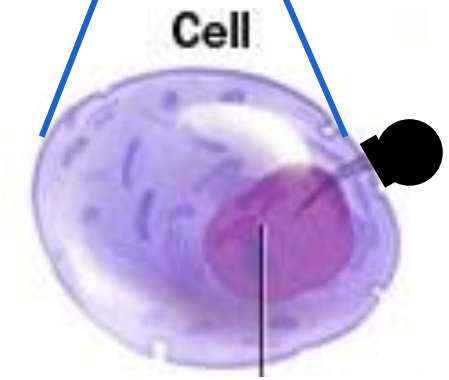
11 maart 2023

De cel

The New Me in 80 Days

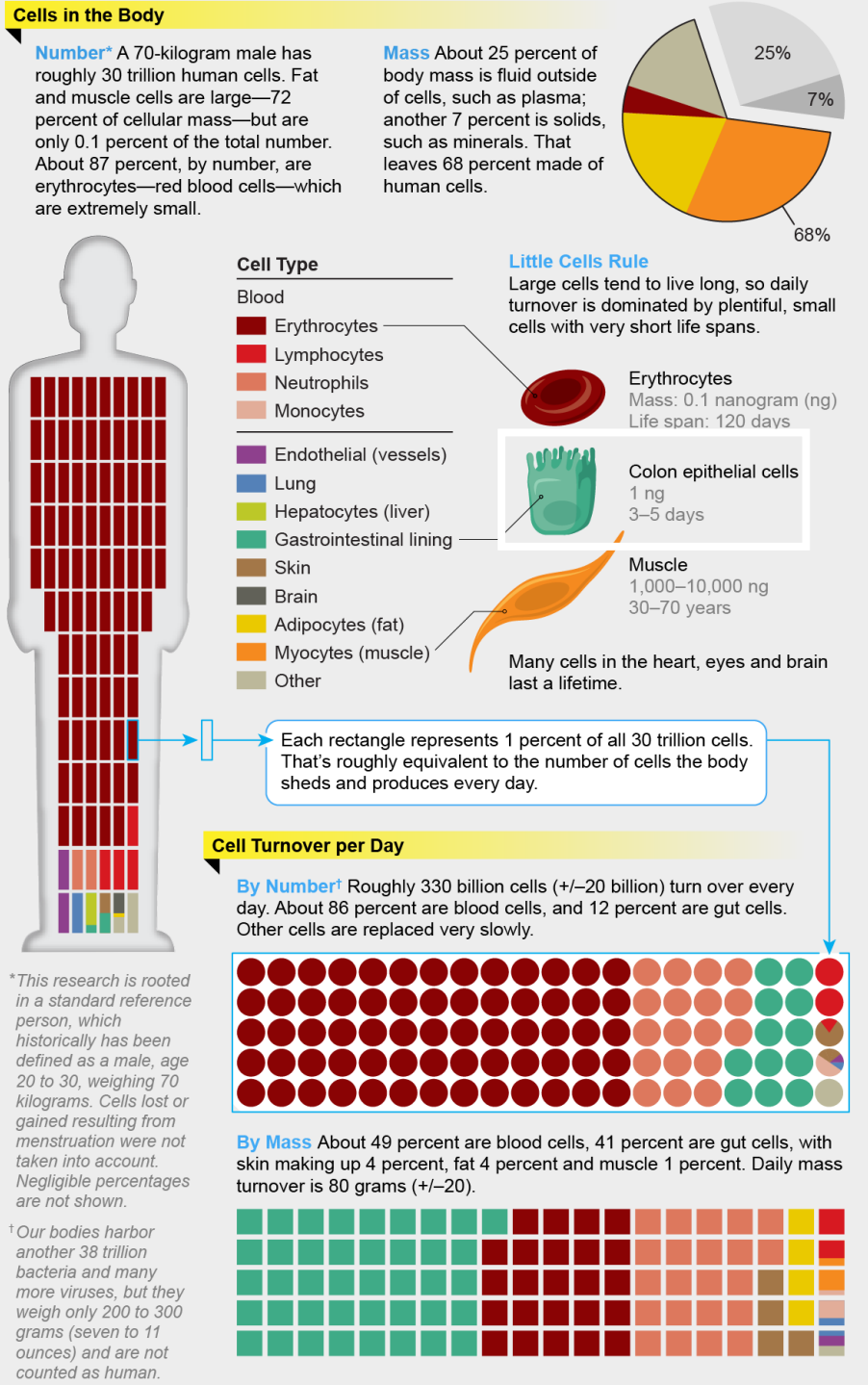
The race to replace the cells in my body!



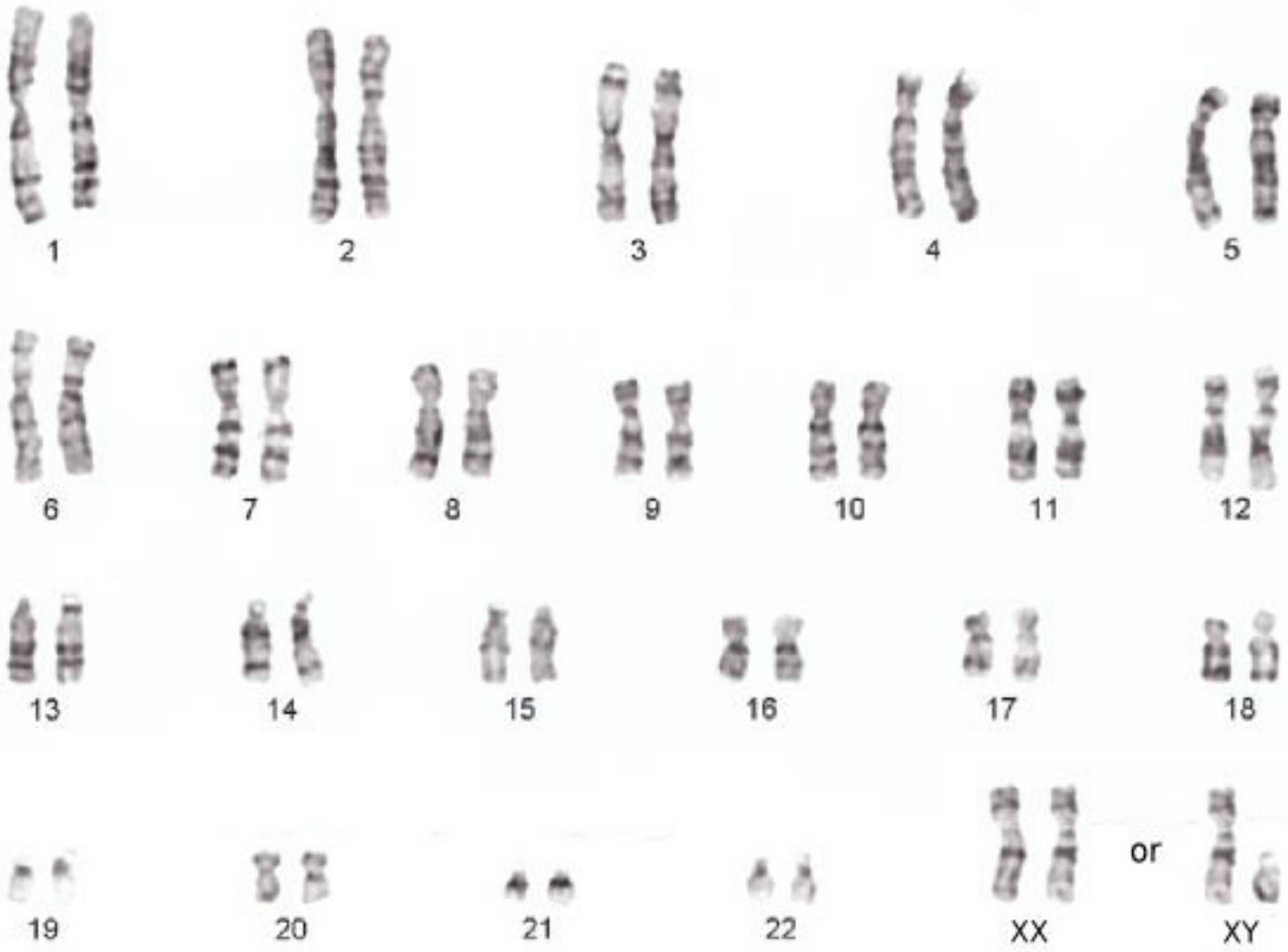


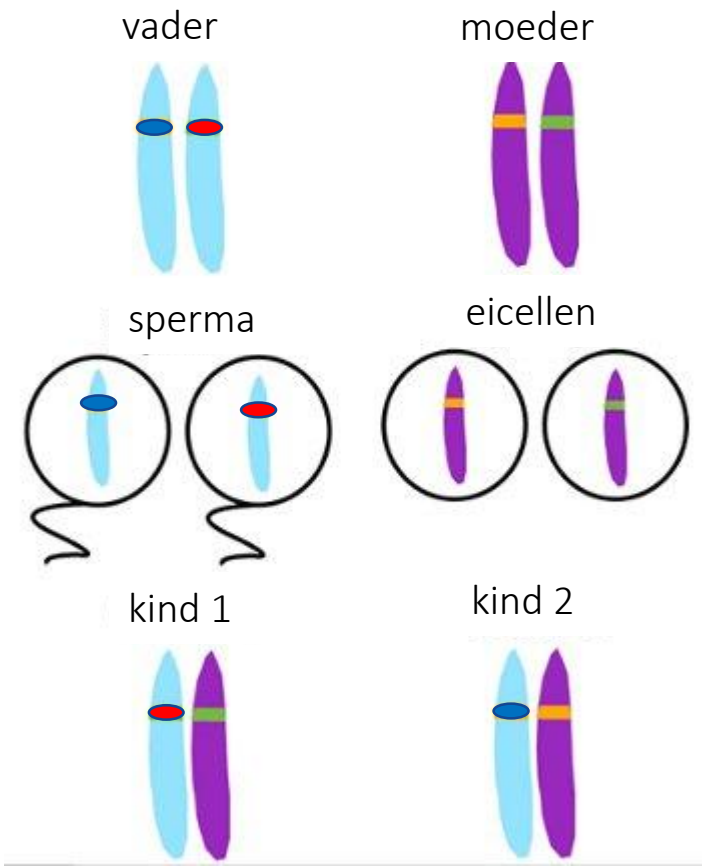
Nucleus = celkern

30 triljoen cellen (30.10^{12}) in een menselijk lichaam

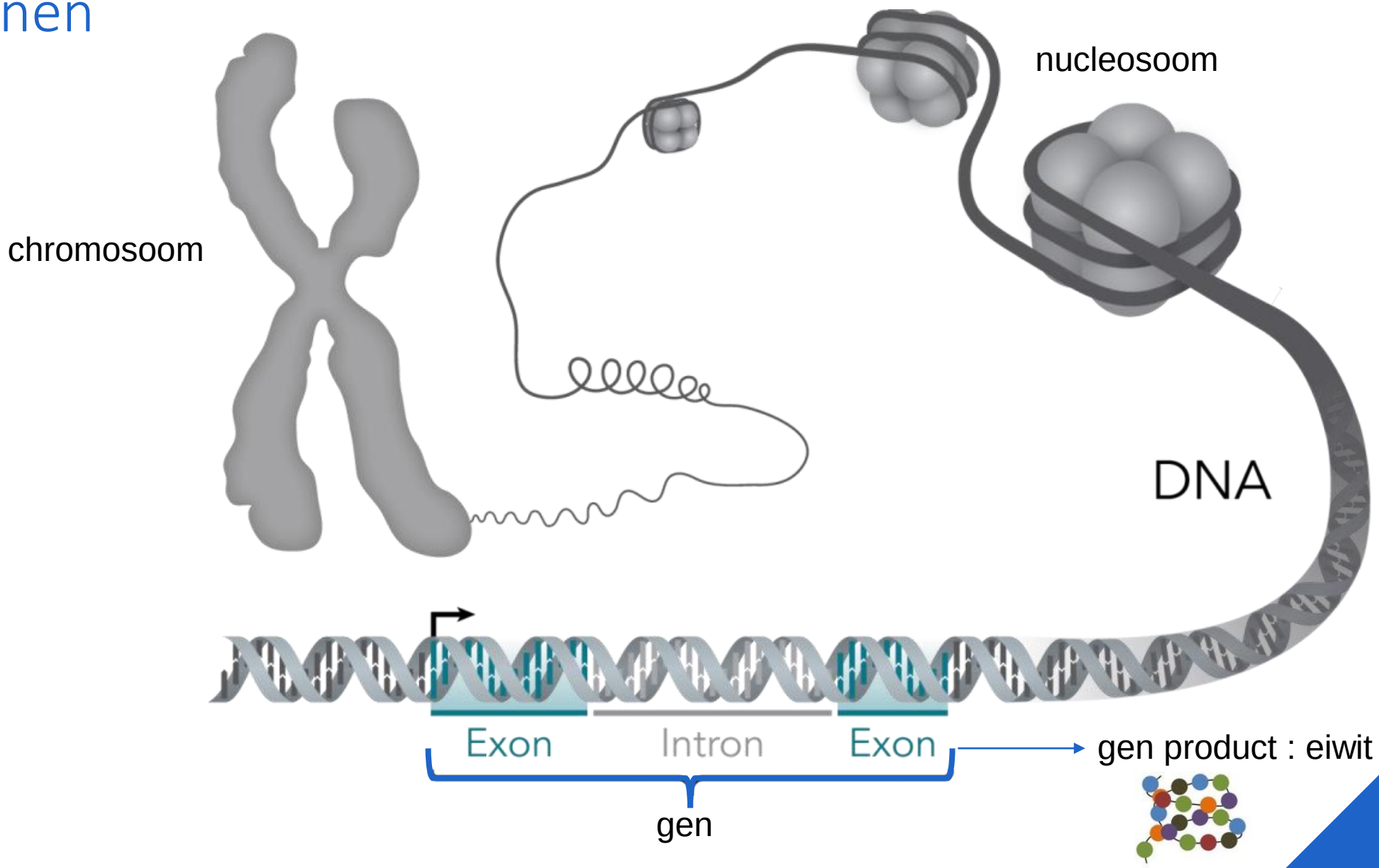


Het humane genoom: 23 paar chromosomen

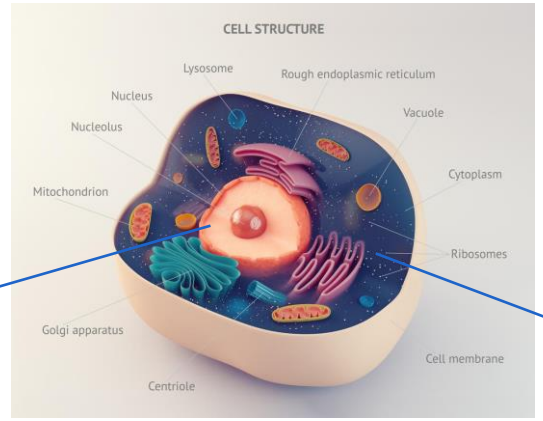




Onze genen

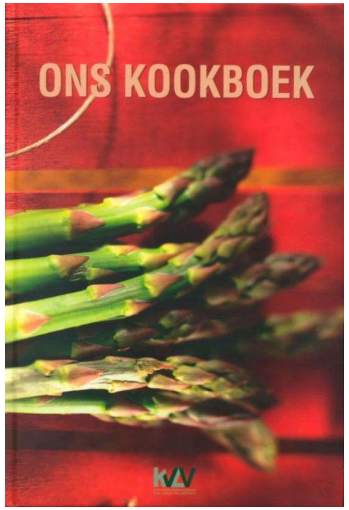

chromosoom
nucleosoom
DNA
Exon
Intron
Exon
gen
gen product : eiwit

Het centrale dogma van de moleculaire biologie

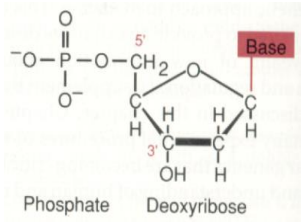


celkern

cytoplasma

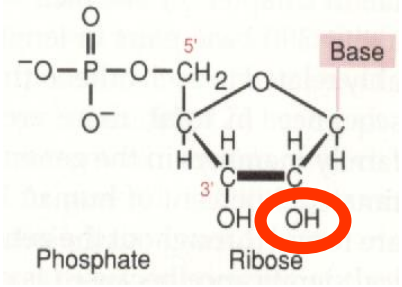


DNA



Transcriptie

mRNA

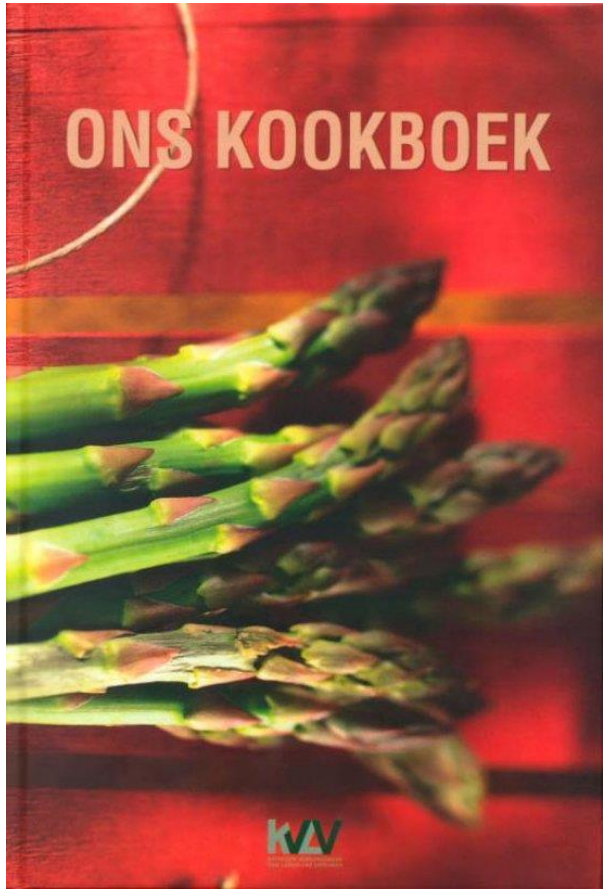


Translatie

Eiwit

Replicatie

Veranderingen in het genoom



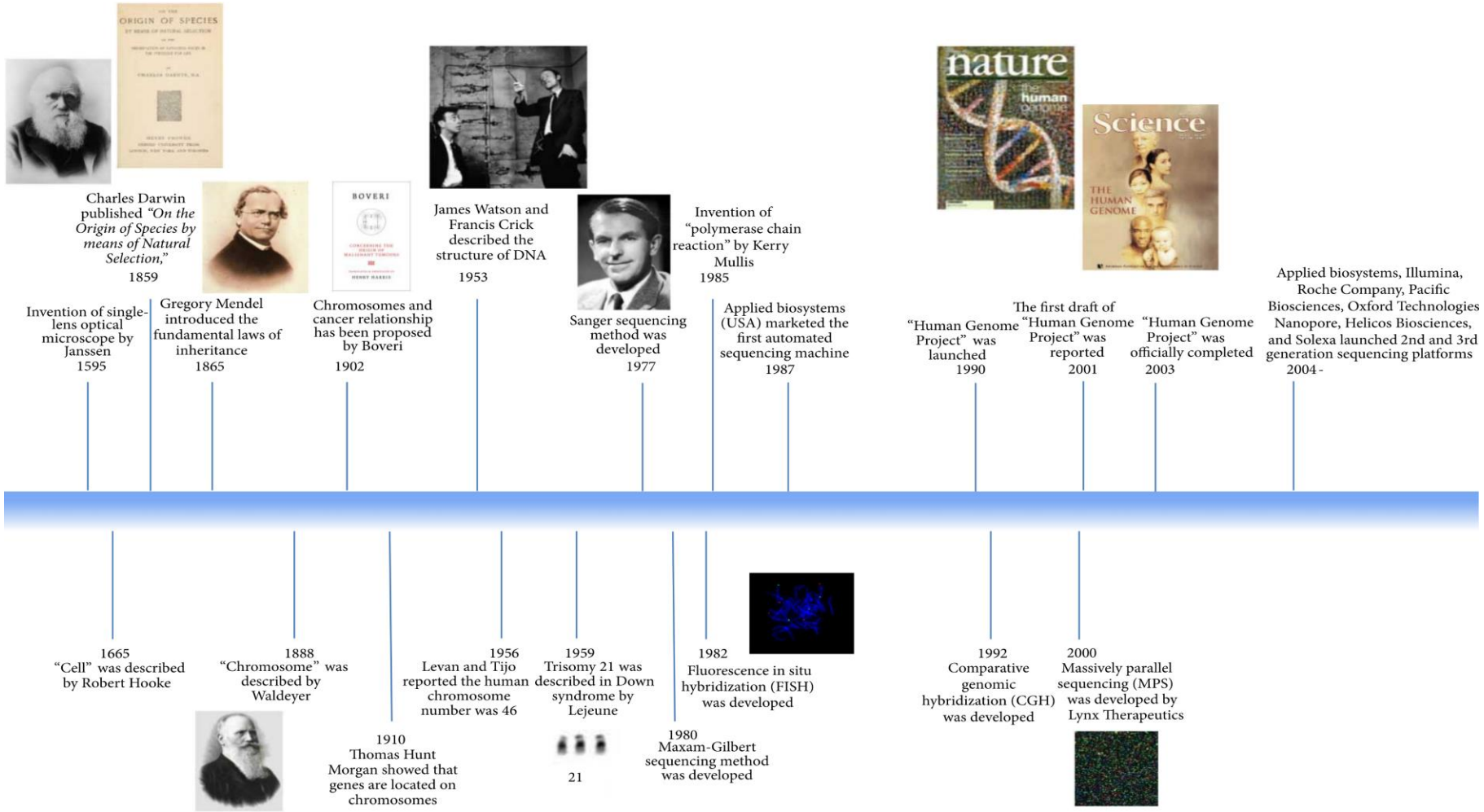
“...maak de bereiding van het product lichtjes zouter door...”

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

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

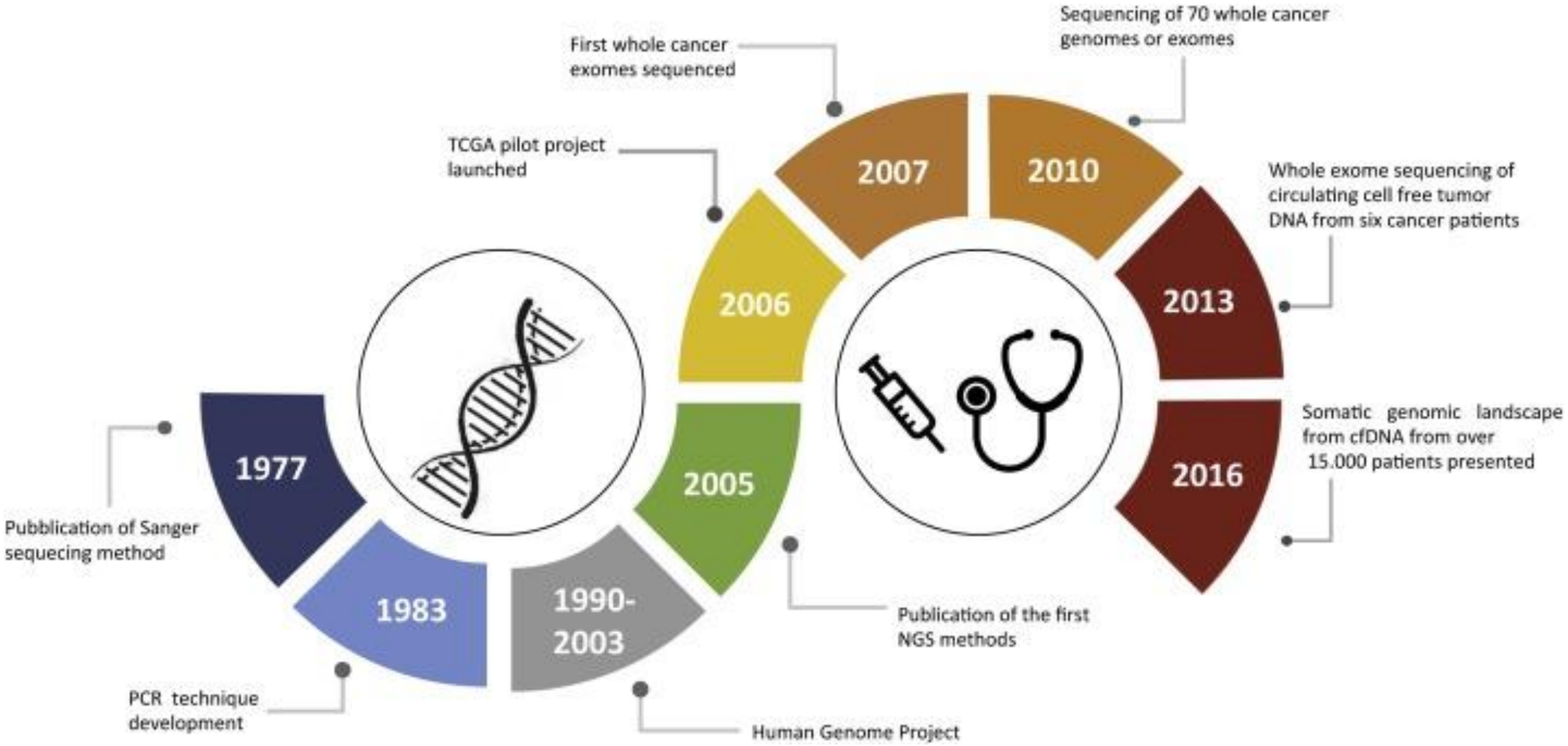
“...maak de bereiding van het product lichtjes zoeter door...”

Historiek genetische technieken



ASUDE ALPMAN DURMAZ, EMIN KARACA, URSZULA DEMKOW, GOKCE TORUNER, JACQUELINE SCHOUMANS, OZGUR COGULU, "EVOLUTION OF GENETIC TECHNIQUES: PAST, PRESENT, AND BEYOND", *BIOMED RESEARCH INTERNATIONAL*, VOL. 2015, ARTICLE ID 461524, 7 PAGES, 2015. HTTPS://DOI.ORG/10.1155/2015/461524

Mijlpalen…



10 / MORGANTI ET AL. CRIT REV ONCOL HEMATOL. 2019 JAN;133:171-182.

Het humane genoom

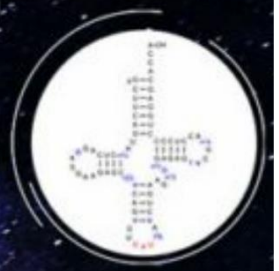
4 bouwstenen: A, C, G, T
3 miljard letters

A Journey Through The History Of DNA Sequencing

Fun and Facts | Science News

2. November 2020

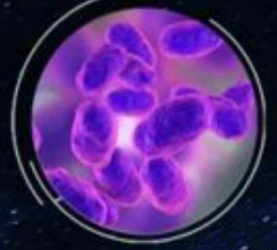
1869
iedrich Mieschet
solates DNA, in
the form of
chormatin



1965
Robert Holley
and colleagues
sequence yeast
tRNA

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

1984
Fritz Pohl develops
NON-RADIOACTIVE
sequencing
platform

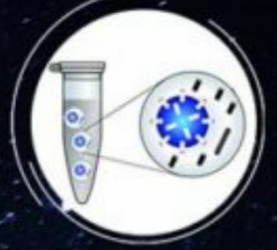


1995
genome sequenced:
H. INFLUENZAE

1998
Solexa develops
"sequencing-by-synthesis"
method that uses
orescent dyes

C. ELEGANS genome

2002
Complete
MOUSE
genome is



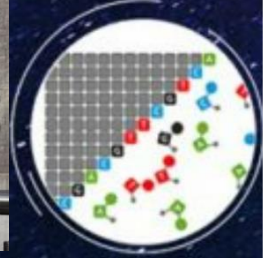
2007
SOLiD system,
which uses
SEQUENCING BY
LIGATION,
is launched

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

1953
ck, Watson and
nklin discovered
e structure of
DNA as a
UBLE 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
irst complete genom
bacteriophage øx17



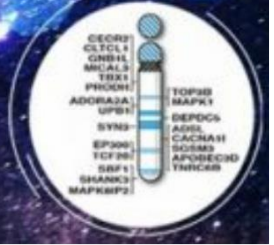
1987
Leroy Hood and
ABI develop first
semi-automated
DNA sequencing
machines

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

ABI introduces first
commercial
sequencing using
capillary
electrophoresis

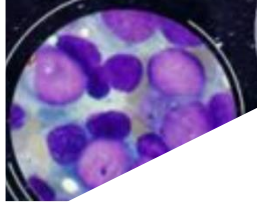
S. CEREVESIAE
genome is
sequenced

1999
First human
chromosome 22
is sequenced



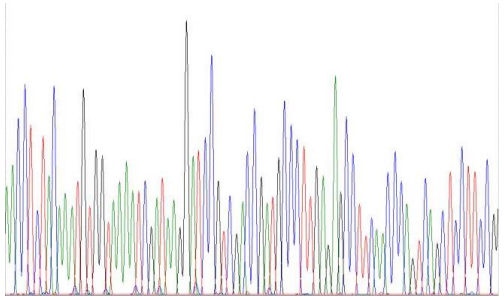
2005
The 454 SYSTEM,
based on
pyrosequencing,
becomes the first
next-generation
sequencer to come
on the market

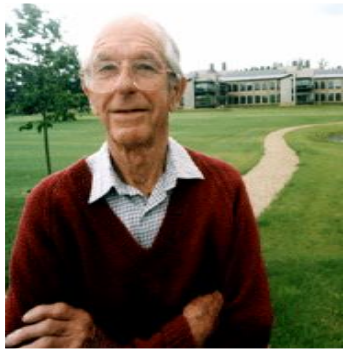
2008
Whole DNA
sequence of a
CANCER is
decoded for
the first time



2012
Oxford Nanopore
Technologies
commercializes
NANOPORE
SEQUENCING

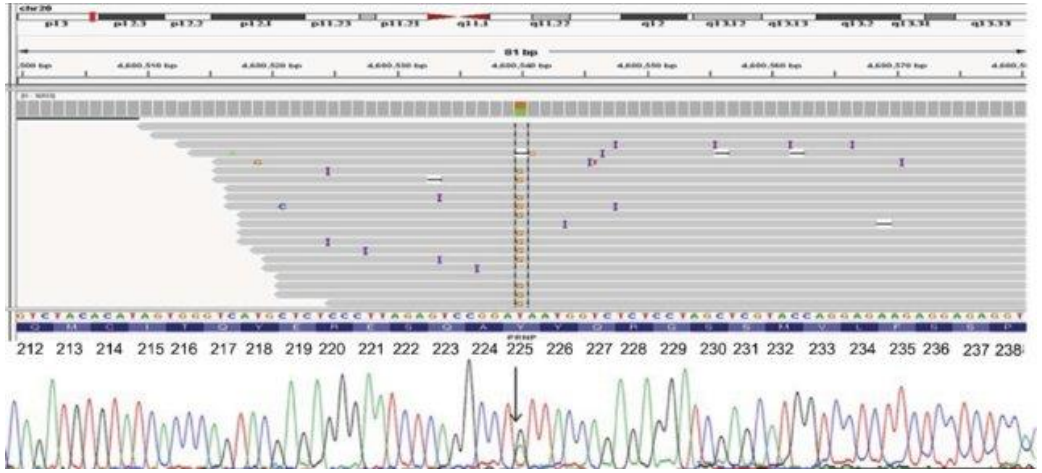
Sequenering – grote evolutie





Fred Sanger Nobel
price 1980

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

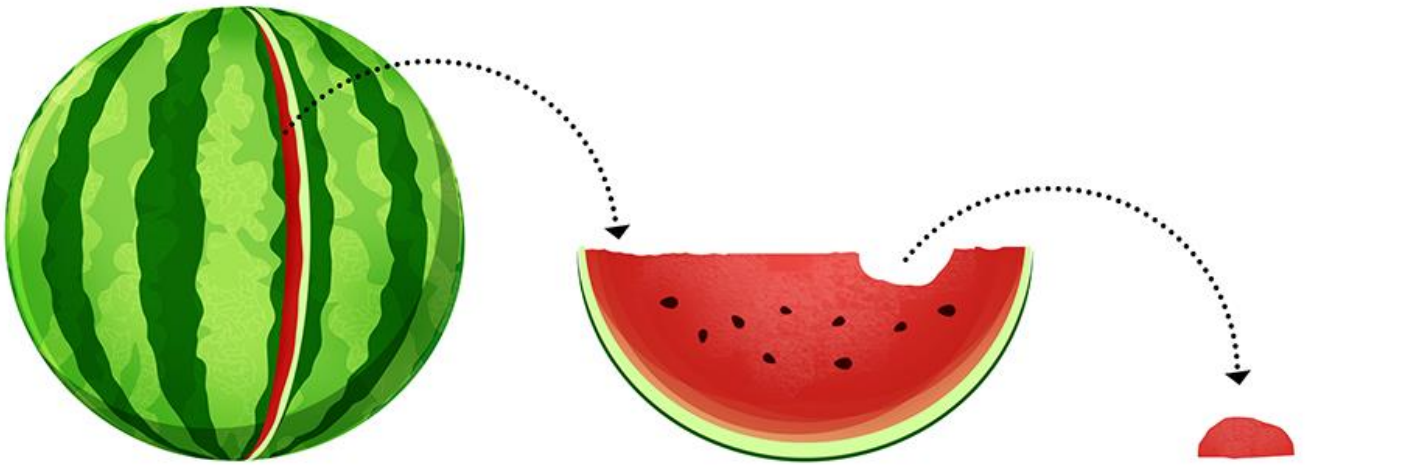


20 000 genen
250 Euro
‘1 week’

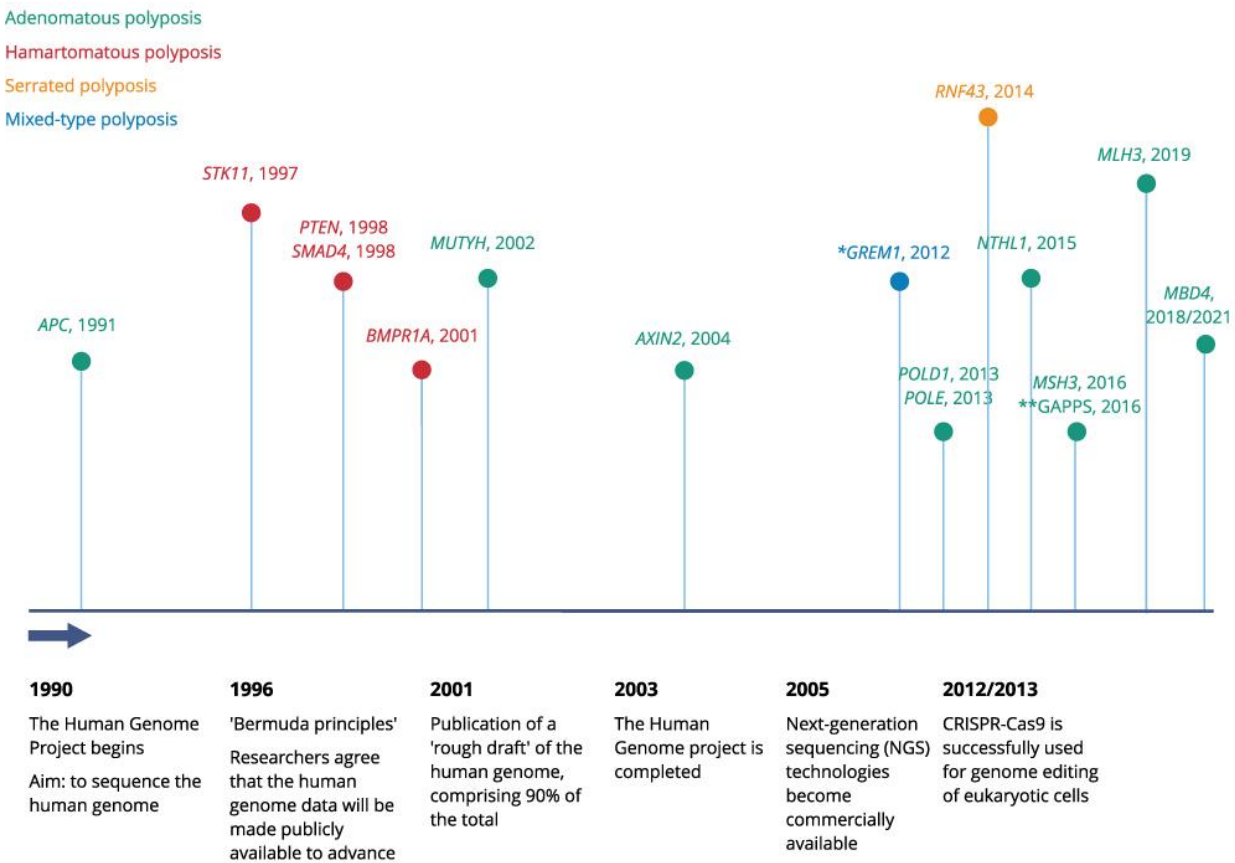
Sanger Sequencing

Next-generation sequencing

Genetische diagnostiek: types van genetische testen



polyposis

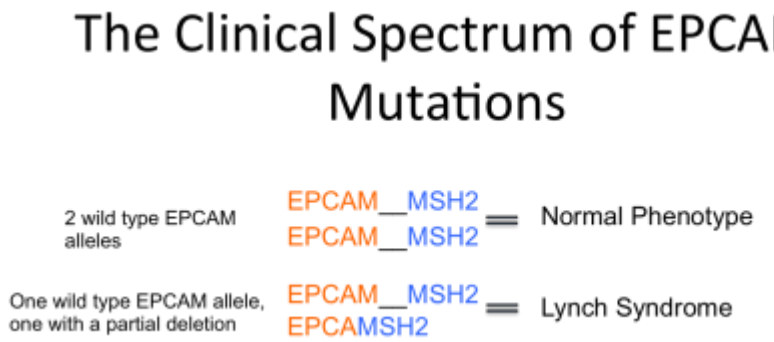


HISTOLOGY	GENE (CLINICAL ENTITY)	INHERITANCE	PATHWAY
Adenomatous	*APC* (familial adenomatous polyposis, FAP)*	Autosomal dominant	WNT/β-catenin signaling pathway
	AXIN2 (oligodontia-colorectal cancer syndrome)		WNT/β-catenin signaling pathway
	POLD1 (polymerase proofreading-associated polyposis, PPAP)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

JELSIG AM, BYRJALSEN A, BUSK MADSEN M, KUHLMANN TP, VAN OVEREEM HANSEN T, WADT KAW, KARSTENSEN JG. NOVEL GENETIC CAUSES OF GASTROINTESTINAL POLYPOSIS SYNDROMES. *APPL CLIN GENET*. 2021;14:455-466 HTTPS://DOI.ORG/10.2147/TACG.S295157

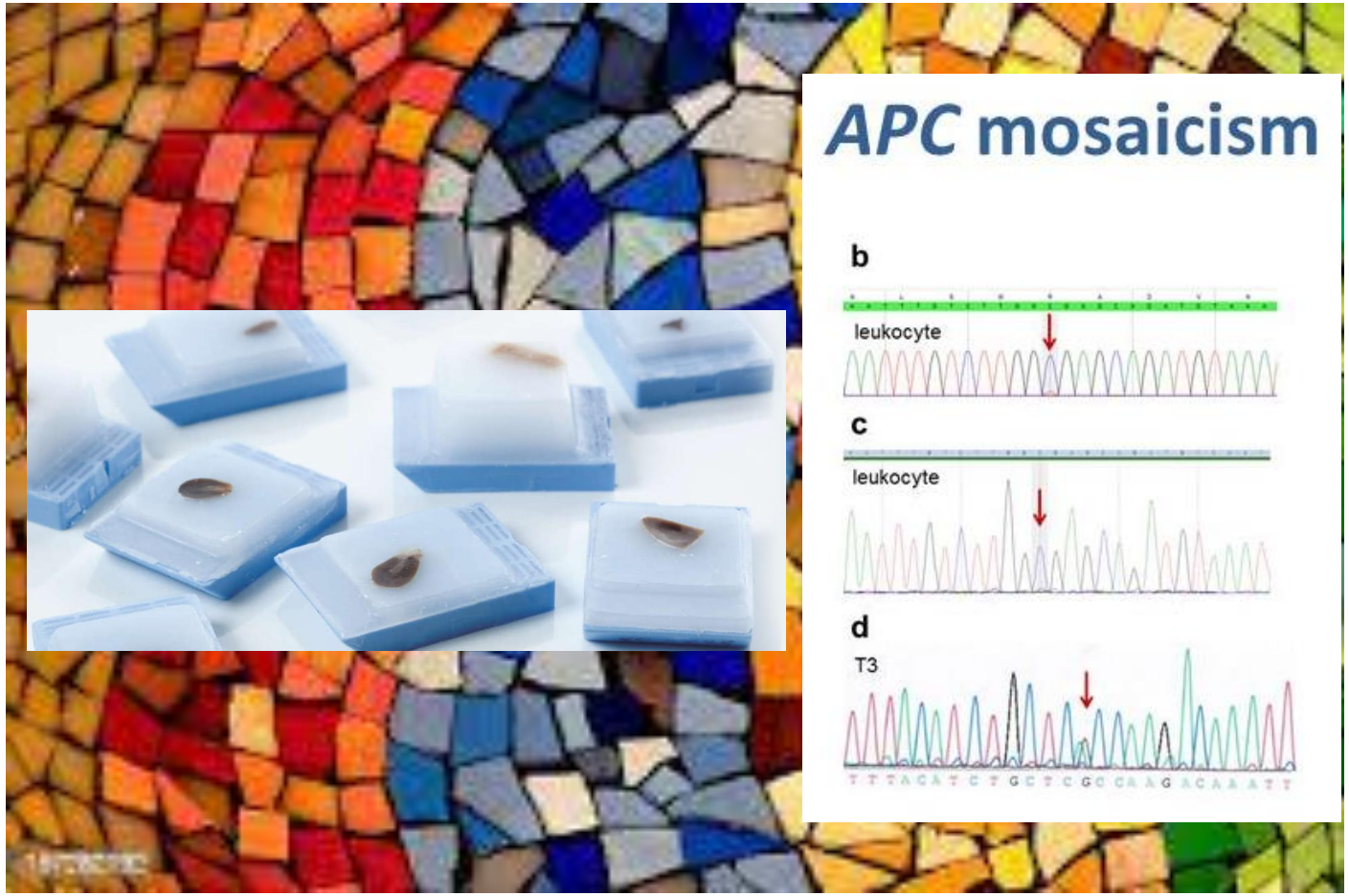
nonpolyposis

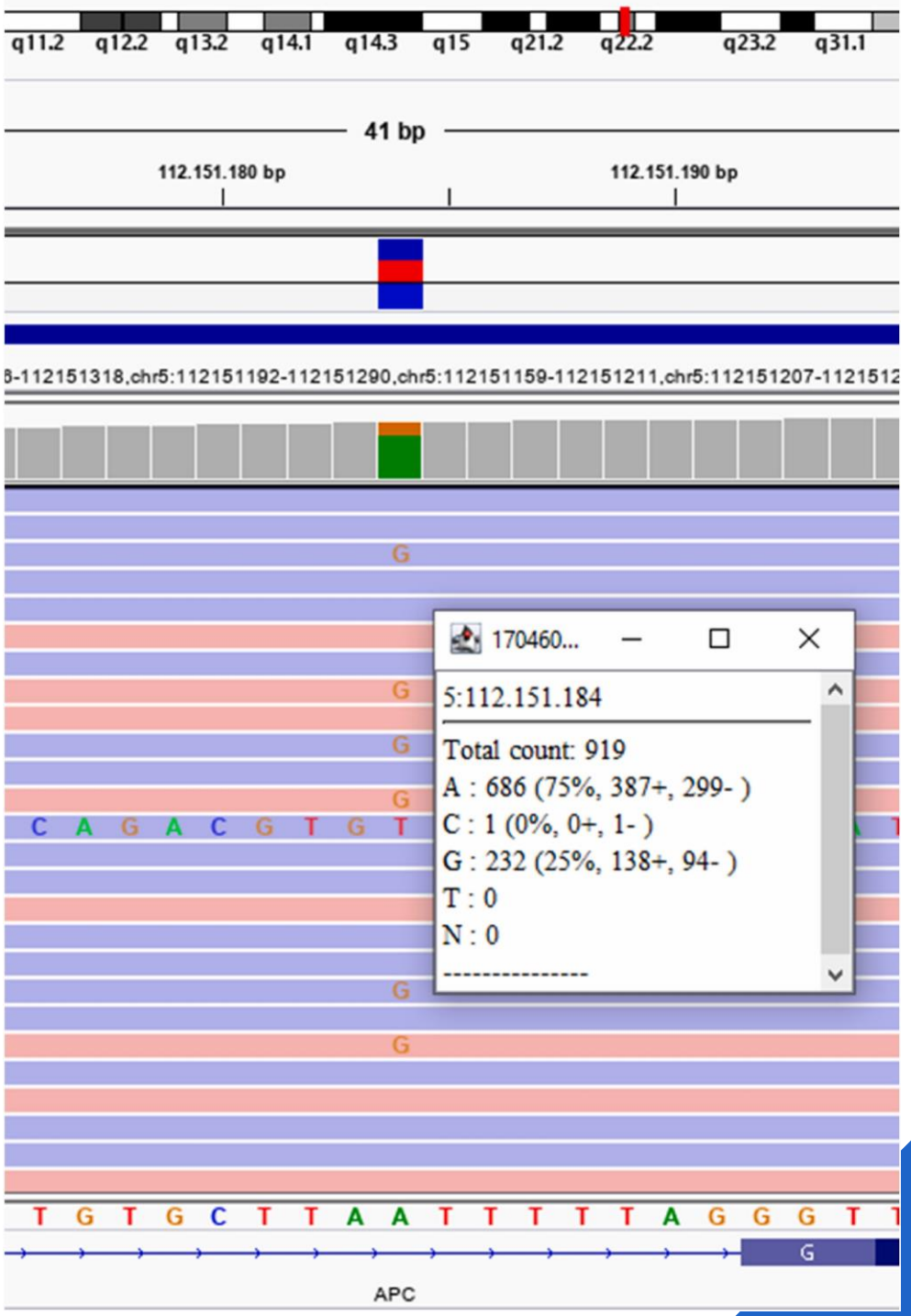
MMR genes	Chromosome Location	Frequency
MSH2	2p16	45-50%
MLH1	3p22	20%
MSH6	2p16	10%
PMS2	7p22	1%
PMS1	2q32	Rare
MSH3	5q14.1	Rare
EXO1	1q43	Rare
Other unknown genes		20-25%
EPCAM	2p16	<1%



Missing genetic variations…

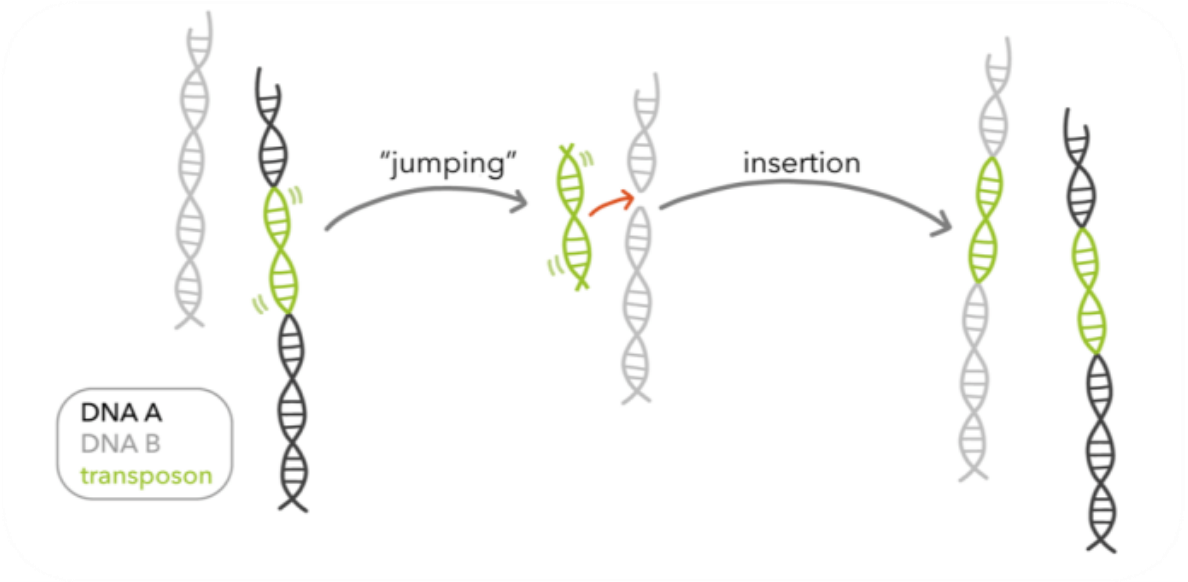
Mozaïeken

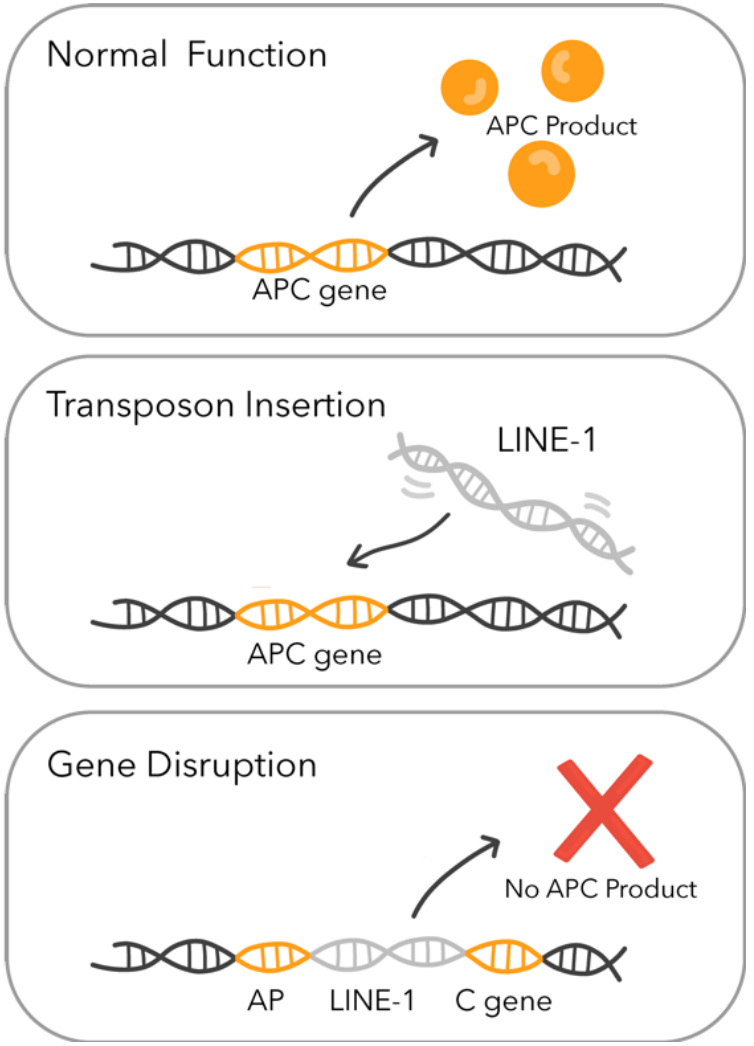




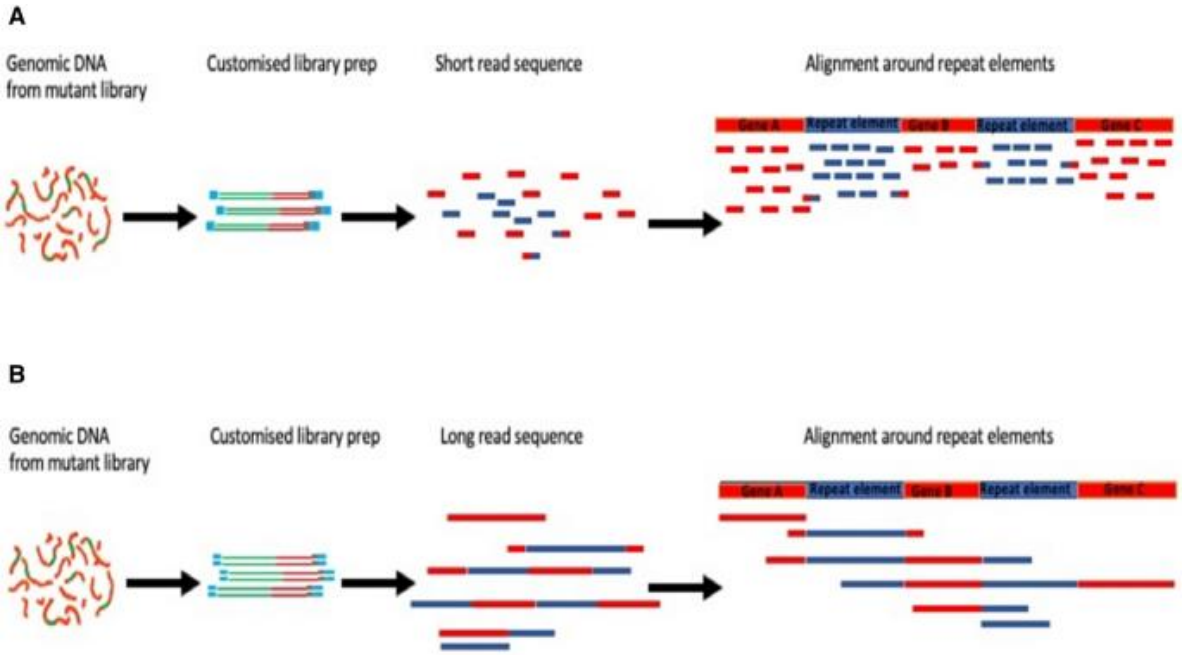
VERENA STEINKE-LANGE, ROBIN DE PUTTER, ELKE HOLINSKI-FEDER, KATHLEEN BM. CLAES, SOMATIC MOSAICS IN HEREDITARY TUMOR PREDISPOSITION SYNDROMES, EUROPEAN JOURNAL OF MEDICAL GENETICS VOLUME 64, ISSUE 12, 2021

“jumping” genes: transposons





Transposons detecteren via long read sequencing



Insertion of an SVA element in MSH2 as a novel cause of **Lynch** syndrome.

3 Cite Share

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

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

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 Cite Share

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

Eur J Hum Genet. 2021 Apr;29(4):680-686. doi: 10.1038/s41431-020-00779-5. Epub 2020 Dec 8.

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 Cite Share

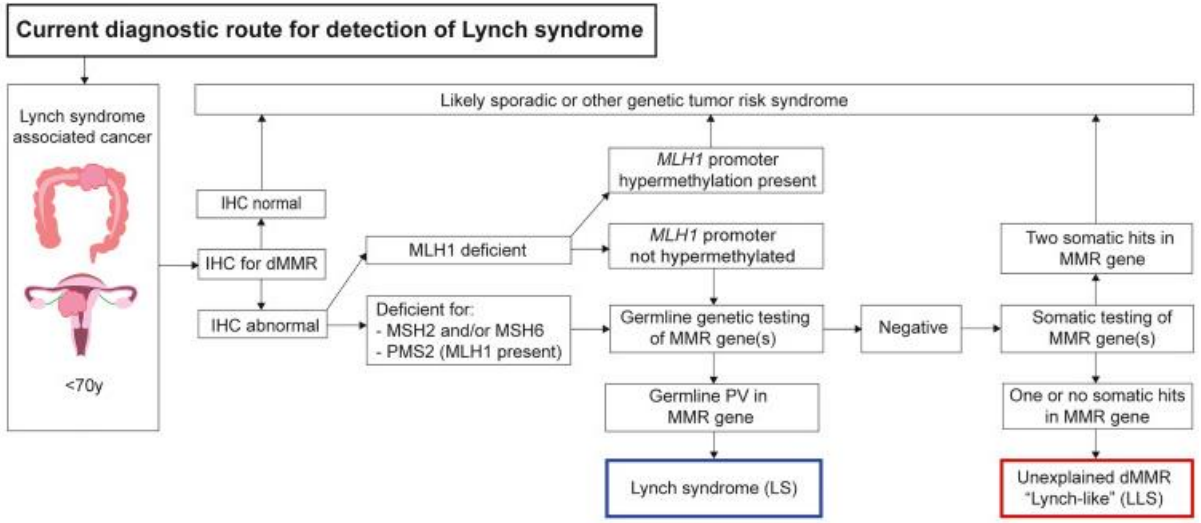
Li Y, Salo-Mullen E, Varghese A, Trottier M, Stadler ZK, Zhang L.

Mol Genet Genomic Med. 2020 Dec;8(12):e1523. doi: 10.1002/mgg3.1523. Epub 2020 Oct 15.

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



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 •

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

PDF + Supplementary Material

Florentine Scharf [1], Rafaela Magalhaes Leal Silva [1], Monika Morak [1], Alex Hastie [2], Julia M A Pickl [1], Kai Sendelbach [1], Christian Gebhard [1], Melanie Locher [1], Andreas Laner [1], Verena Steinke-Lange [1], Udo Koehler [1], 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@mgz-muenchen.de; Professor Elke Holinski-Feder; elke.holinski-feder@mgz-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.

Variant classificatie

Germline variants: 5 classes

- **>0.99** — Class 5
  - Predicted to be **pathogenic** , this result therefore **confirms** the diagnosis
- **0.90-0.99** — Class 4
  - **Likely** pathogenic, **consistent** with the diagnosis
- **0.05-0.949** — 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.
- **0.001-0.049** — 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?
- **<0.001** — Class 1
  - **Not** pathogenic.
  - "Commom" polymorphism.
  - No evidence suggesting pathogenicity and at "high" frequency.

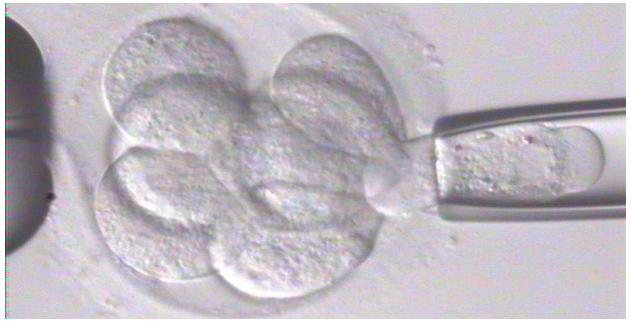
Predictieve test
Prenatale test
PGT

Class (3), 4, 5 are reported

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)

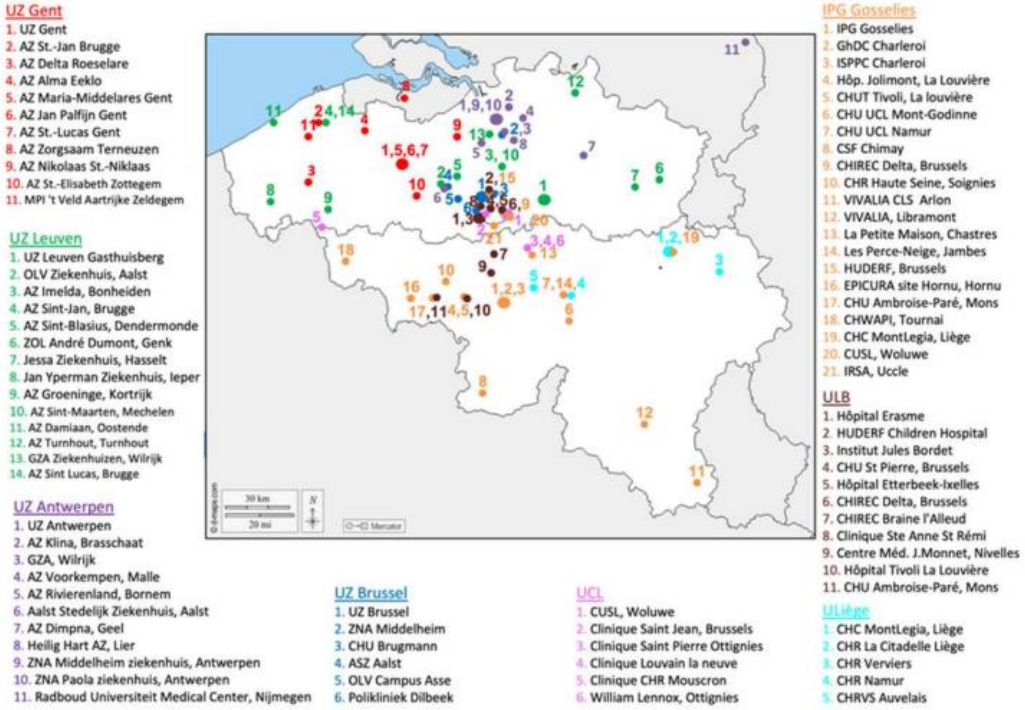


ACGGTCCTTTGAAG
ACGGTCCTTTGAAG

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)



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

Informatie – waar kan je terecht?

European Reference Networks | Genetic Tumour Risk Syndromes (ERN GENTURIS)

Home | About us | Our experts | Patients area | Thematic disease groups | Guidelines and pathways | Research | Education and training | News and events

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 >>

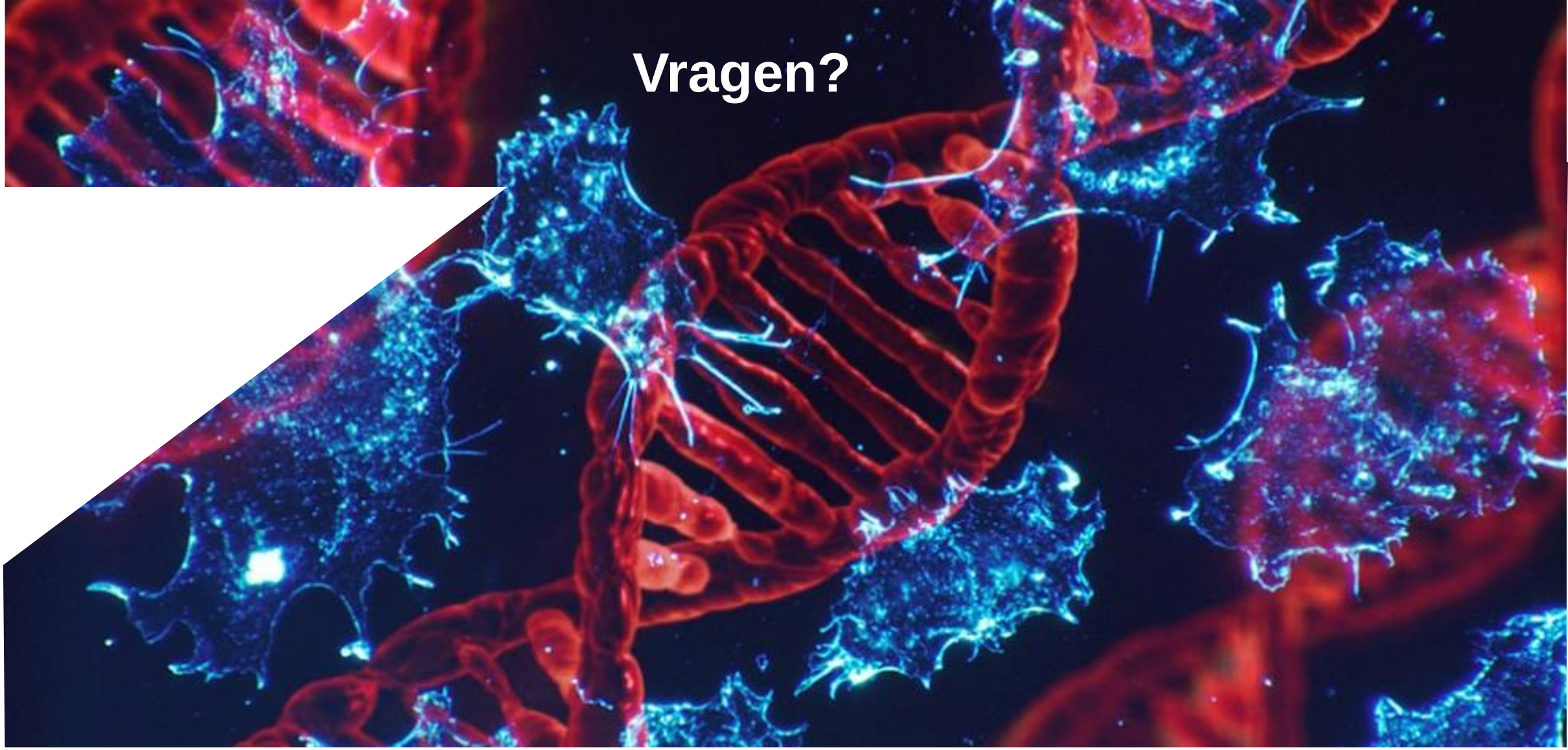
European Reference Networks

Share. Care. Cure.

ERN GENTURIS

With every diagnosis
we can help an entire family

Vragen?



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With every diagnosis
we can help an entire family

PROF DR KATHLEEN CLAES

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