Diagnostics in Heritable Connective Tissue Diseases

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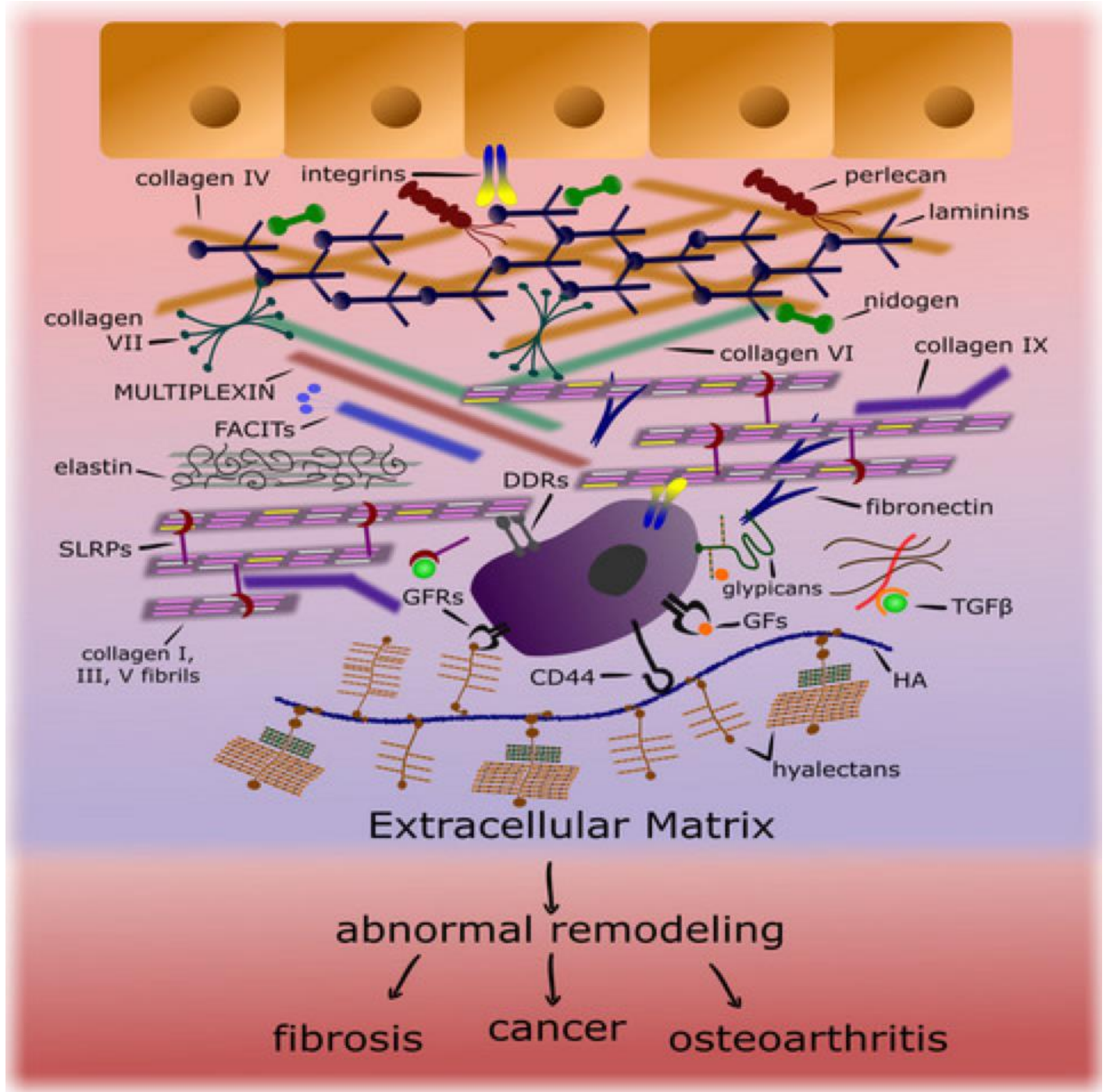
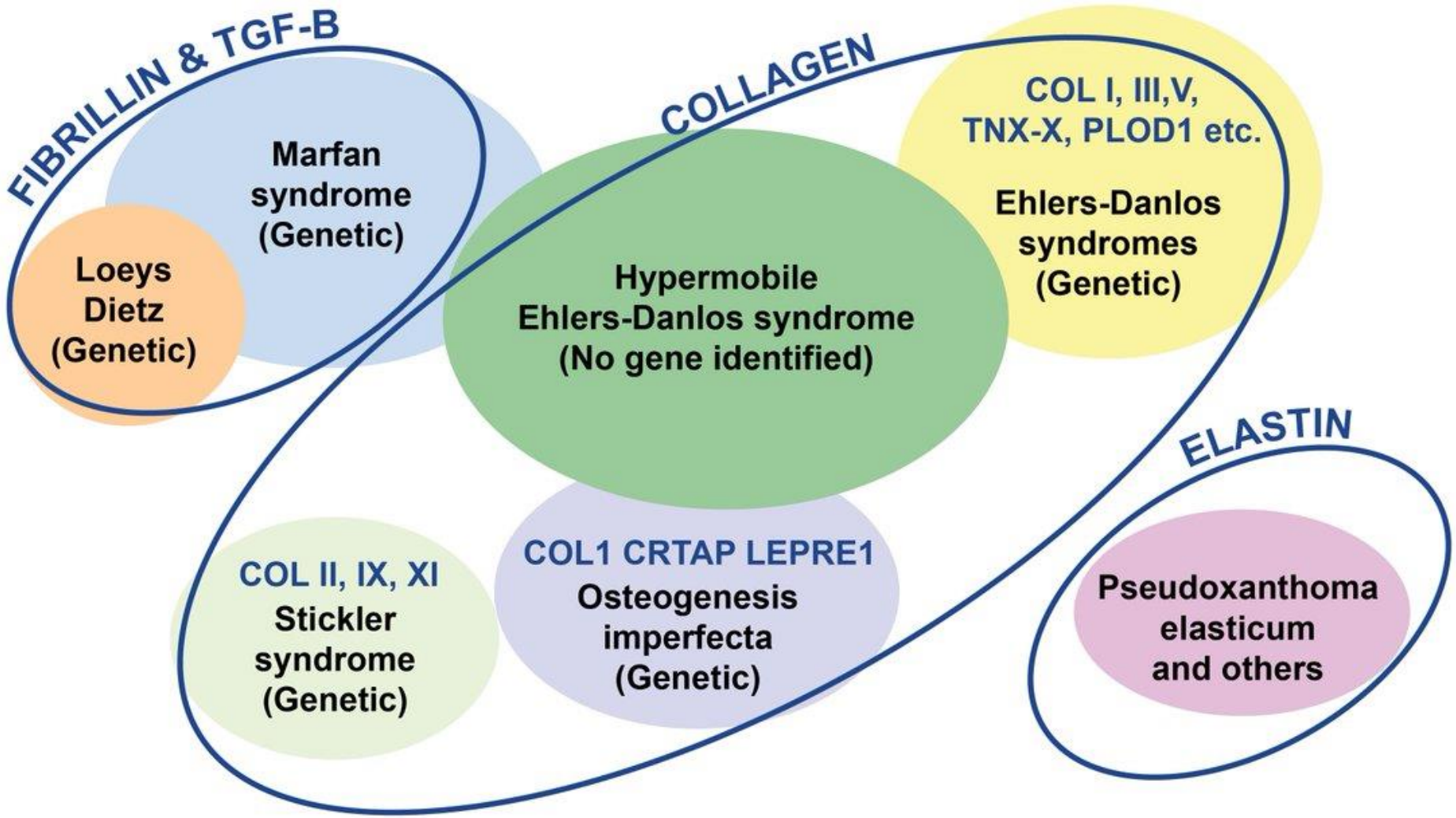
12/11/2024


collagen IV
integrins
perlecan
laminins
nidogen
collagen VII
MULTIPLEXIN
collagen VI
collagen IX
FACITs
elastin
DDRs
fibronectin
SLRPs
GFRs
glypicans
TGFβ
GFs
collagen I, III, V fibrils
CD44
HA
hyalectans
Extracellular Matrix
abnormal remodeling
fibrosis
cancer
osteoarthritis

HMSA
Hypermobility Syndromes Association

The More Common Heritable Disorders of Connective Tissue

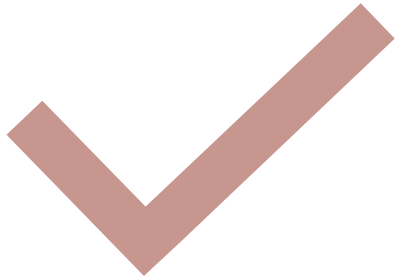


hypermobility.org

Diagnostics in HCTD

- CMGG: https://www.cmgg.be/nl/zorgverlener/labguide/constitutioneel-genetische-aandoeningen
- Belgian Genetic Tests database: https://gentest-acc.healthdata.be/

Diagnostics in HCTD

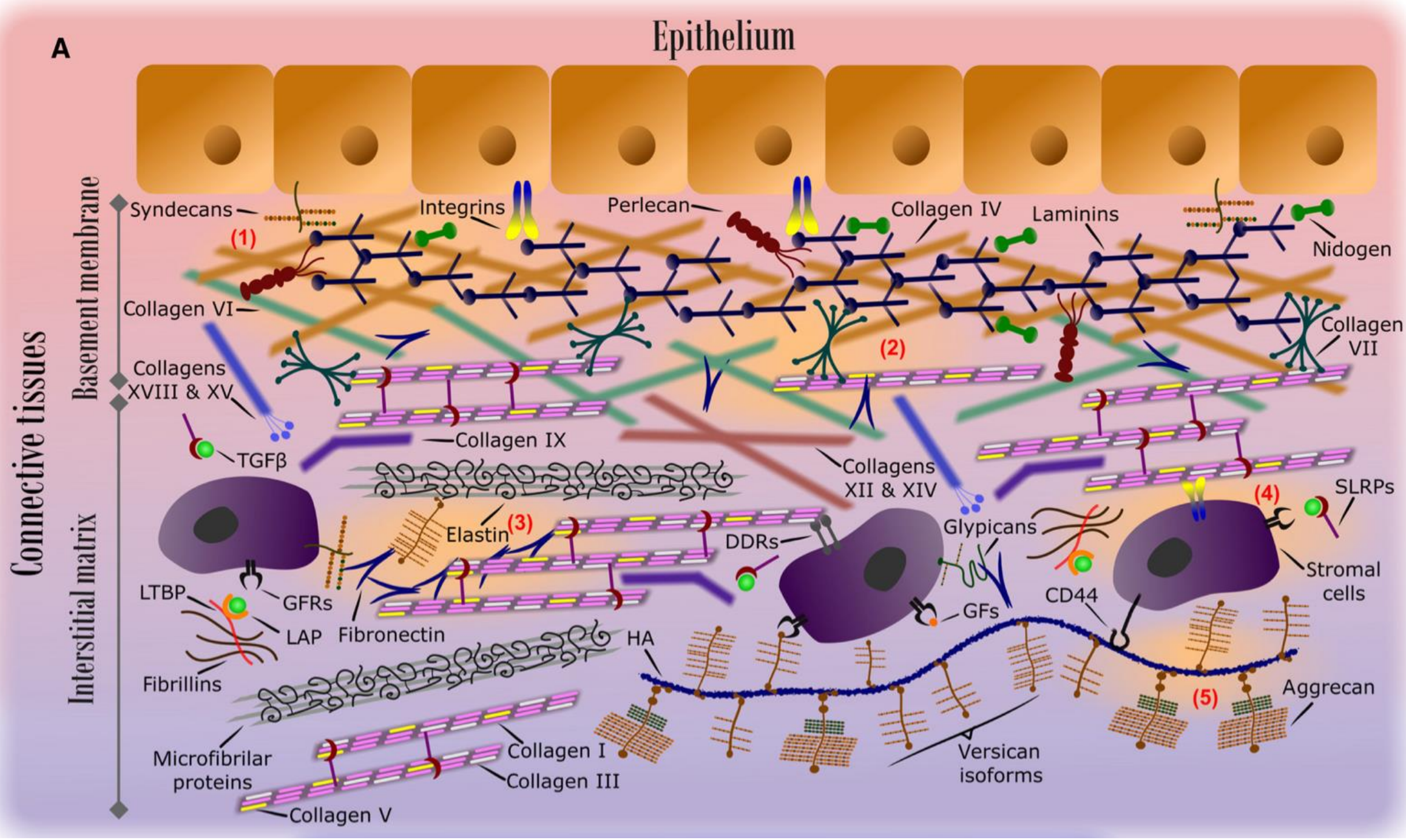


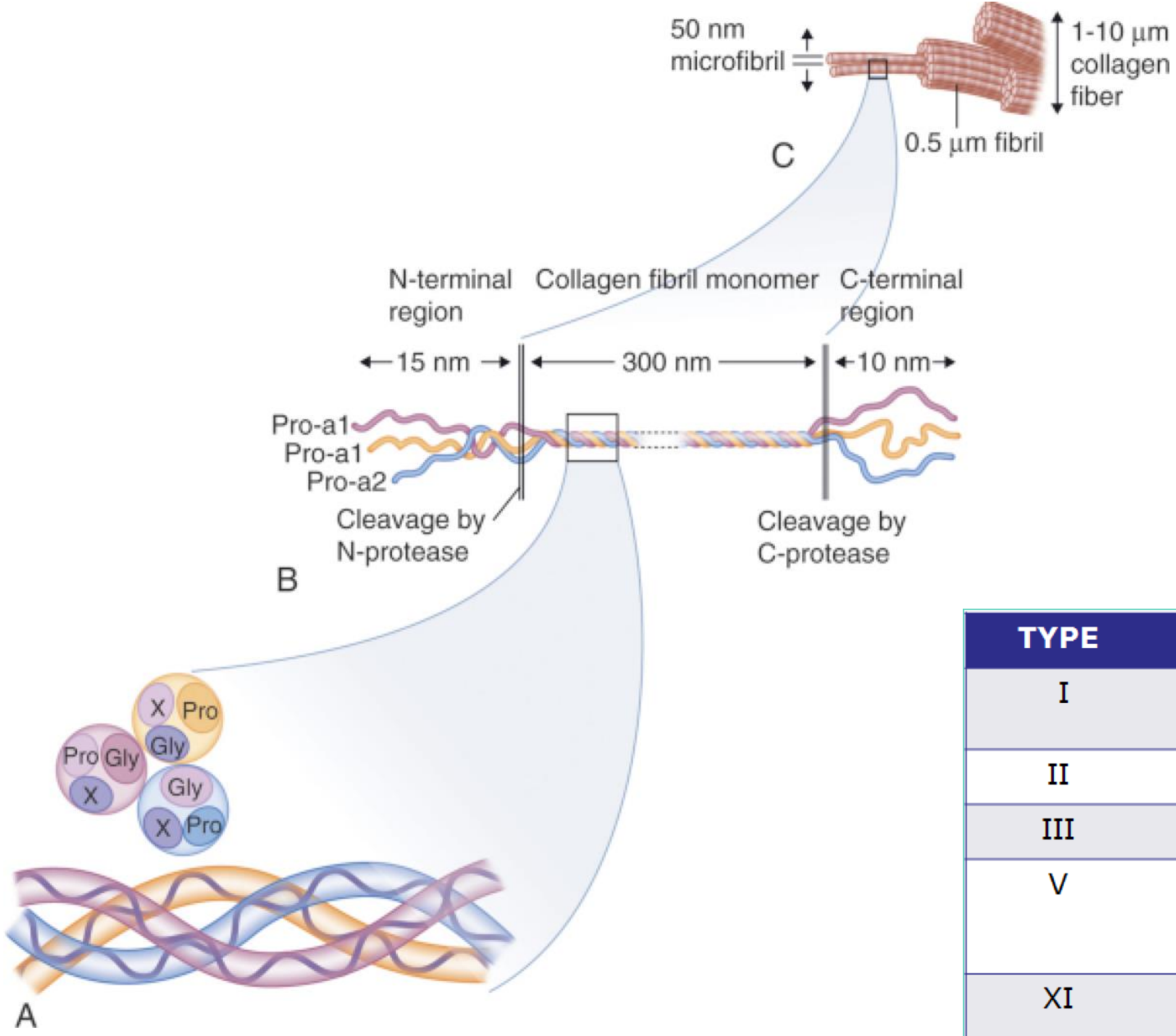
Previously

Current strategies

Previously

- EDS, OI
  - skin biopsy,
  - biochemical collagen analysis,
  - mRNA isolation and sequencing at cDNA level (**cave?**)
  - sequencing at gDNA level
- Others
  - sequencing at gDNA level


A
Epithelium
Connective tissues
Basement membrane
Interstitial matrix
Syndecans
(1)
Integrins
Perlecan
Collagen IV
Laminins
Nidogen
Collagen VI
Collagen VII
(2)
Collagens XVIII & XV
TGFβ
Collagen IX
Collagens XII & XIV
SLRPs
(4)
Elastin
(3)
DDRs
Glypicans
Stromal cells
LTBP
GFRs
LAP
Fibronectin
GFs
CD44
HA
Fibrillins
Aggrecan
(5)
Microfibrilar proteins
Collagen I
Collagen III
Versican isoforms
Collagen V



TYPE	CHAINS	GENES	TISSUE
I	α1(I) α2(I)	*COL1A1* *COL1A2*	skin, tendons, arteries, bone
II	α1(II)	*COL2A1*	cartilage, vitreous
III	α1(III)	*COL3A1*	skin, arteries, uterus
V	α1(V) α2(V) α3(V)	*COL5A1* *COL5A2* *COL5A3*	skin, tendons cornea, bone, ligaments
XI	α1(XI) α2(XI)	*COL11A1* *COL11A2*	cartilage
XXIV	α1(XXIV)	*COL24A1*	cartilage, eye
XXVII	α1(XXVII)	*COL27A1*	cartilage

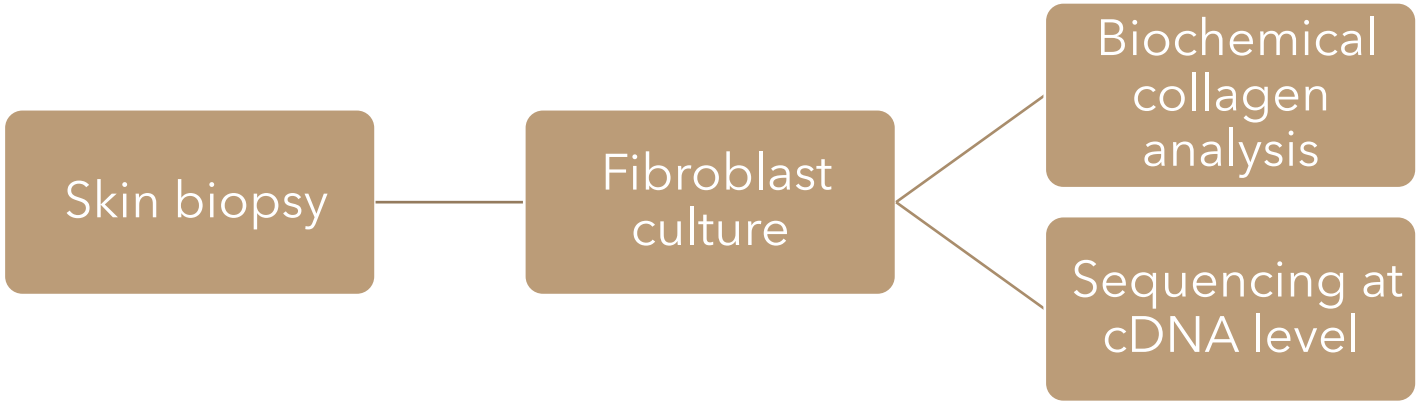
Previously

COLLAGEN

OI

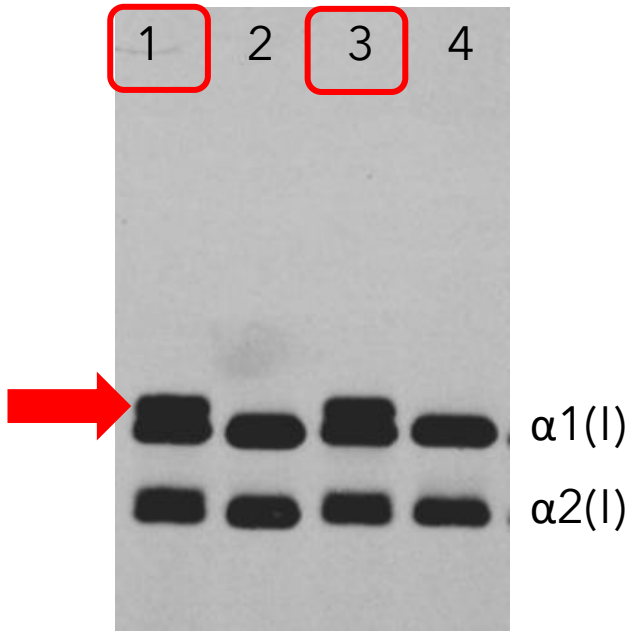
First: protein defect (faster)

Second: genetic defect; gene-by-gene approach

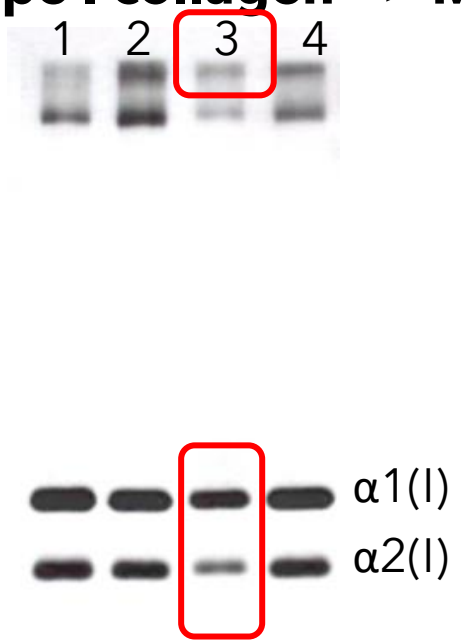


Type I collagen defect - OI

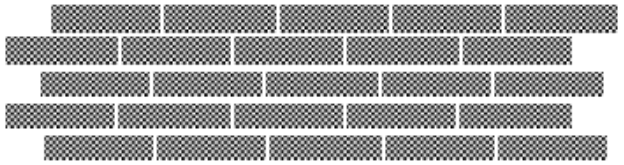
Structurally abnormal type I collagen -
-> Severe/moderate/lethal OI



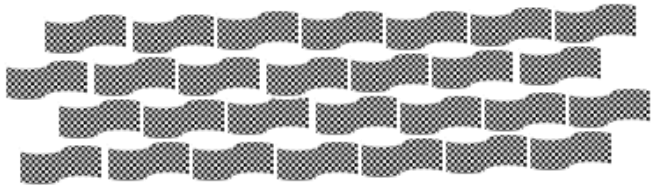
Reduction type I collagen --> Mild OI



Type I collagen defect - OI



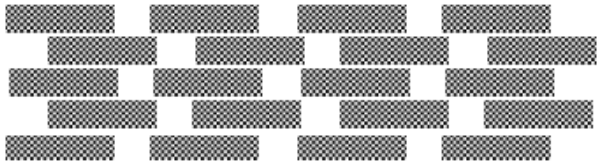
Normal bone structure



Structural defect

Severe/moderate/lethal OI
(type III, IV, II)

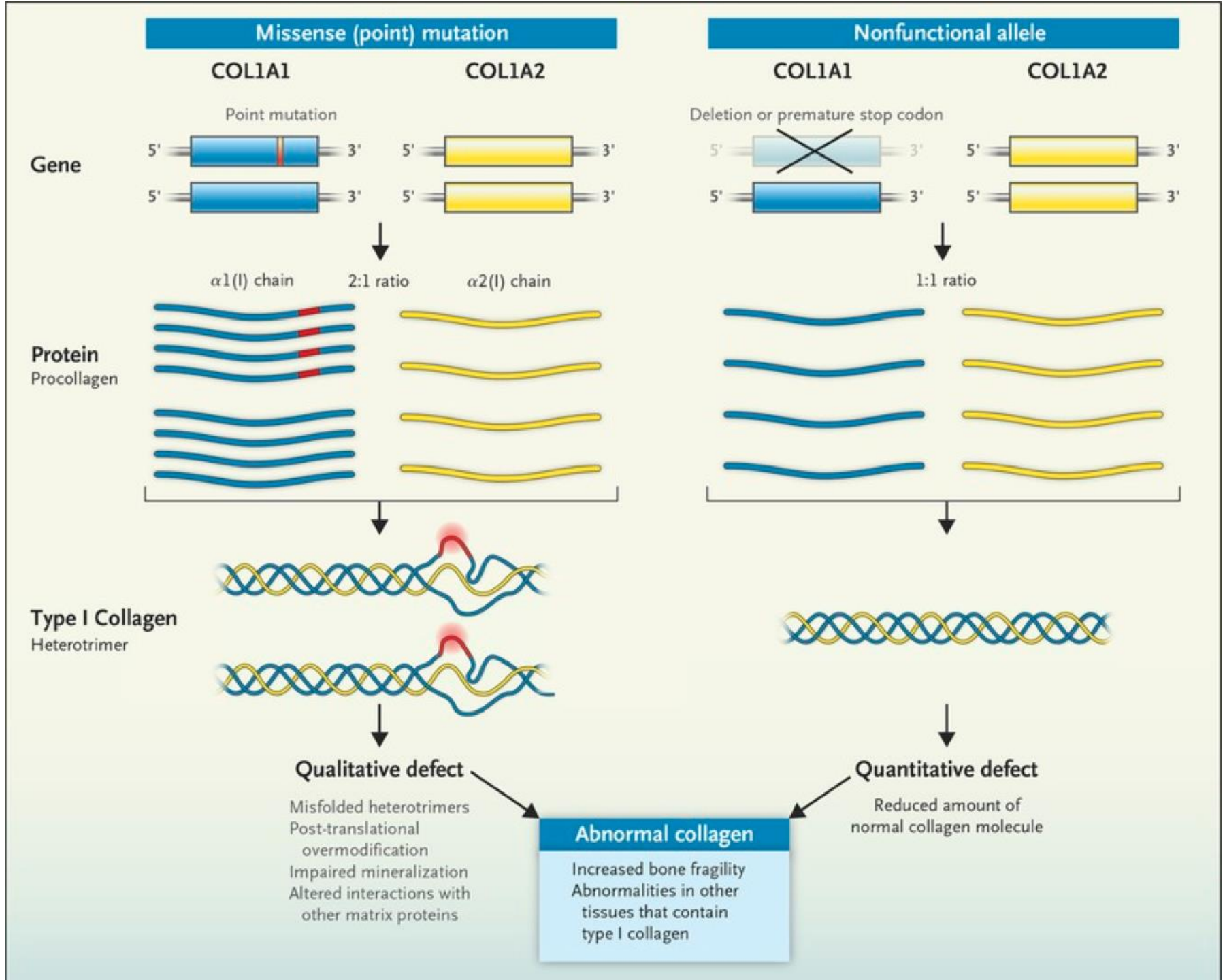
Mainly missense variants

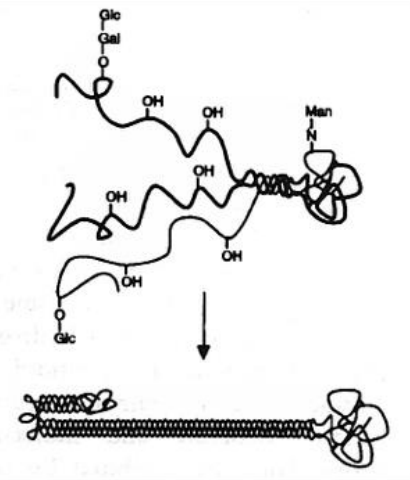


Quantitative defect

Type I (mild) OI

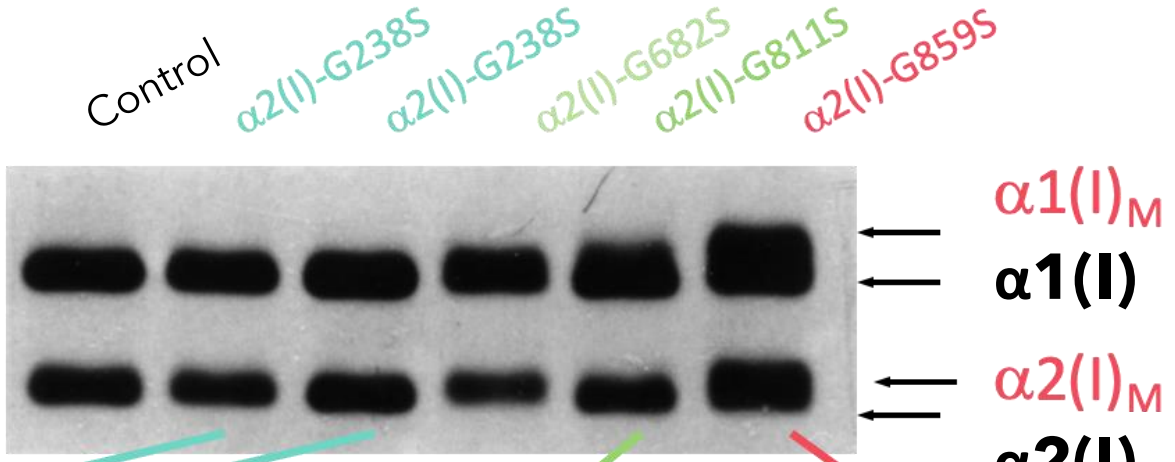
Mainly null-variants

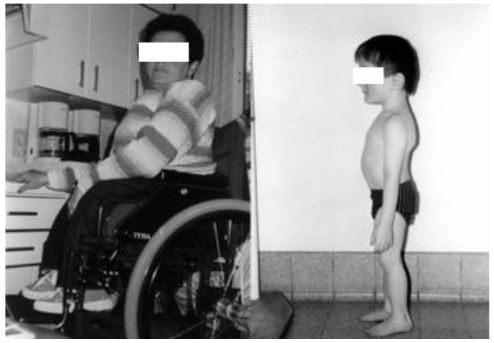

Missense (point) mutation
COL1A1
COL1A2
Point mutation
Nonfunctional allele
COL1A1
COL1A2
Deletion or premature stop codon
Gene
5'
3'
α1(I) chain
2:1 ratio
α2(I) chain
1:1 ratio
Protein
Procollagen
Type I Collagen
Heterotrimer
Qualitative defect
Misfolded heterotrimers
Post-translational overmodification
Impaired mineralization
Altered interactions with other matrix proteins
Quantitative defect
Reduced amount of normal collagen molecule
Abnormal collagen
Increased bone fragility
Abnormalities in other tissues that contain type I collagen



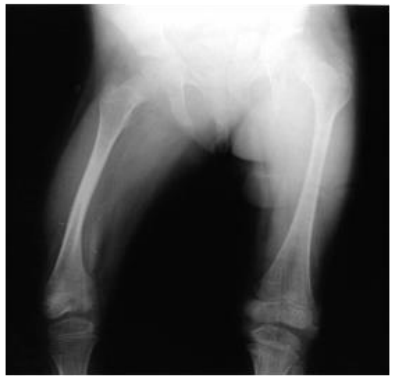
PHENOTYPIC SEVERITY INFLUENCED BY POSITION OF PATHOGENIC VARIANT

Gly>Ser in α2(I) chain

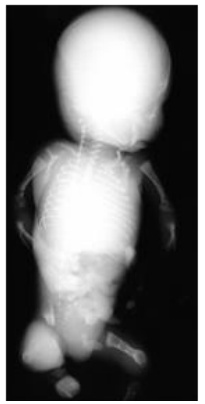




OI type III OI type IV



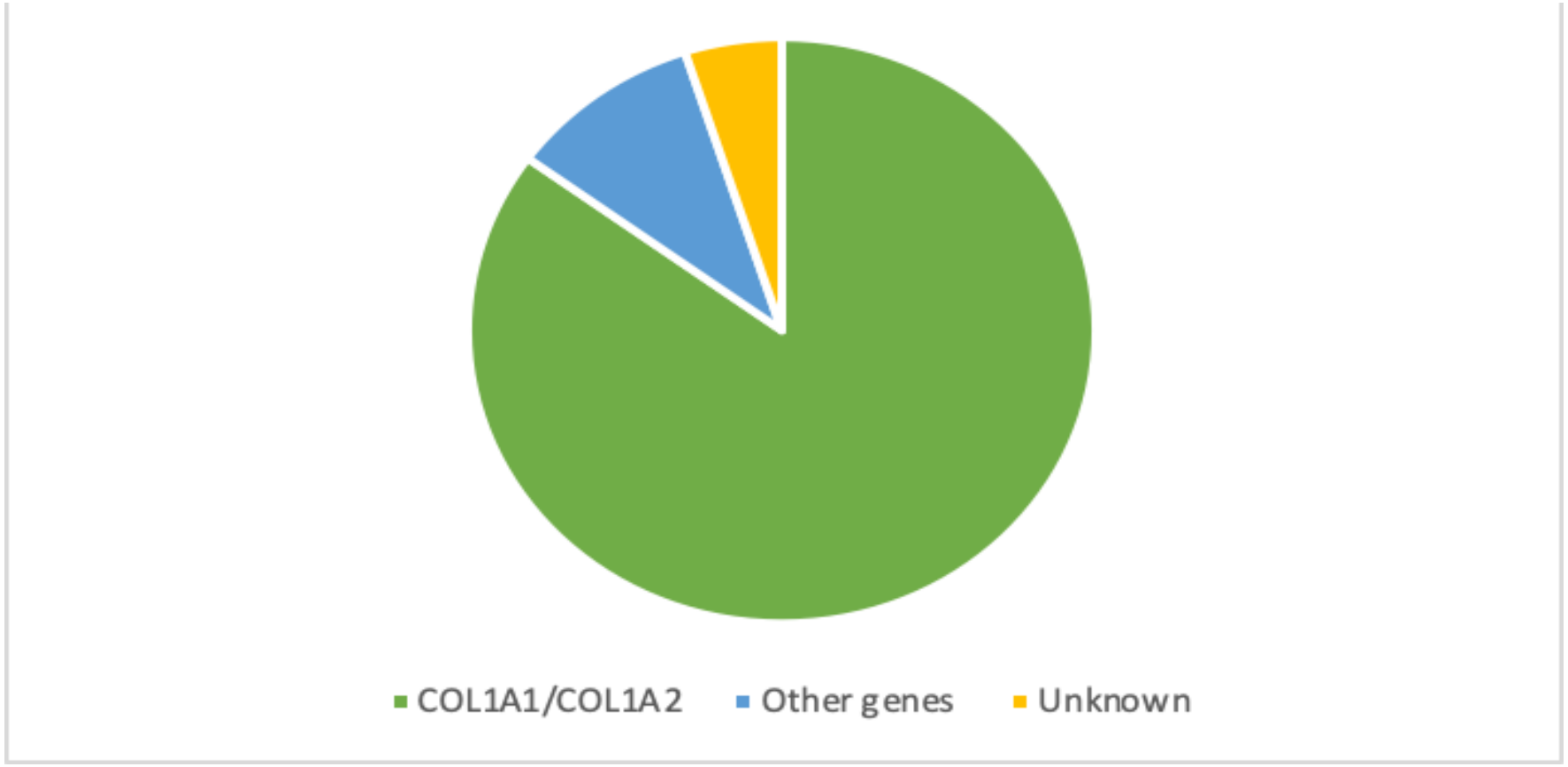
OI type IV



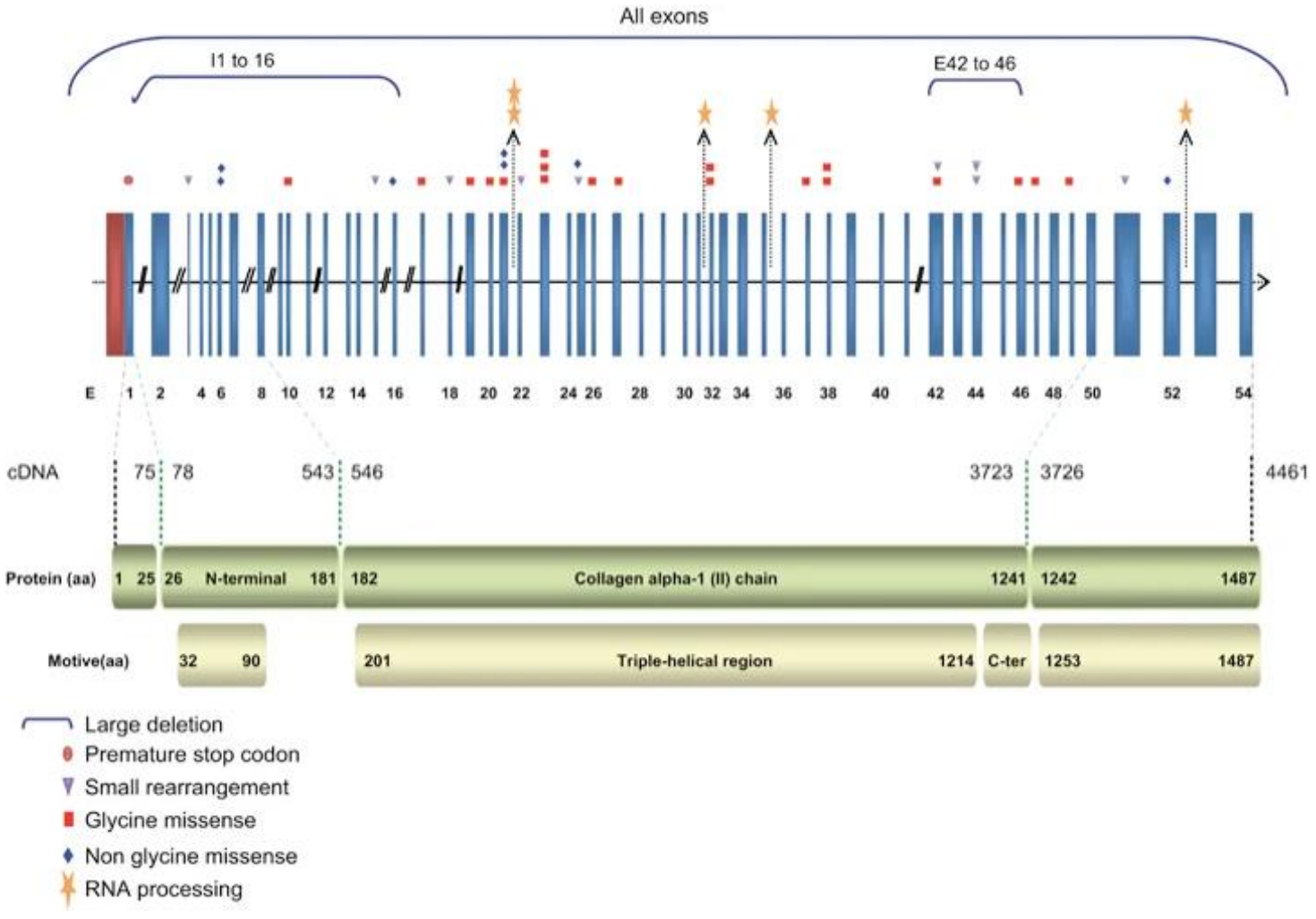
OI type II

Type I collagen - OI

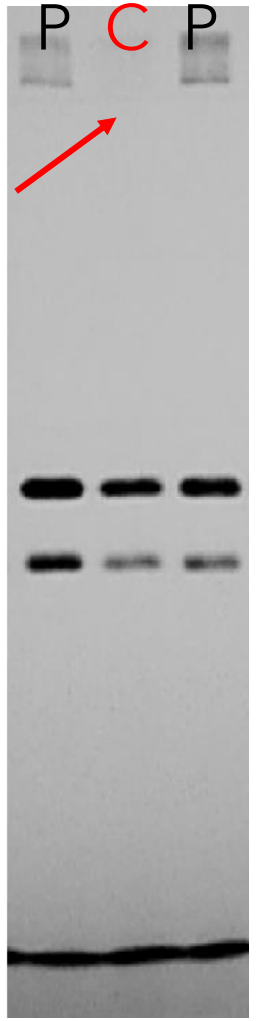
Variant spectrum



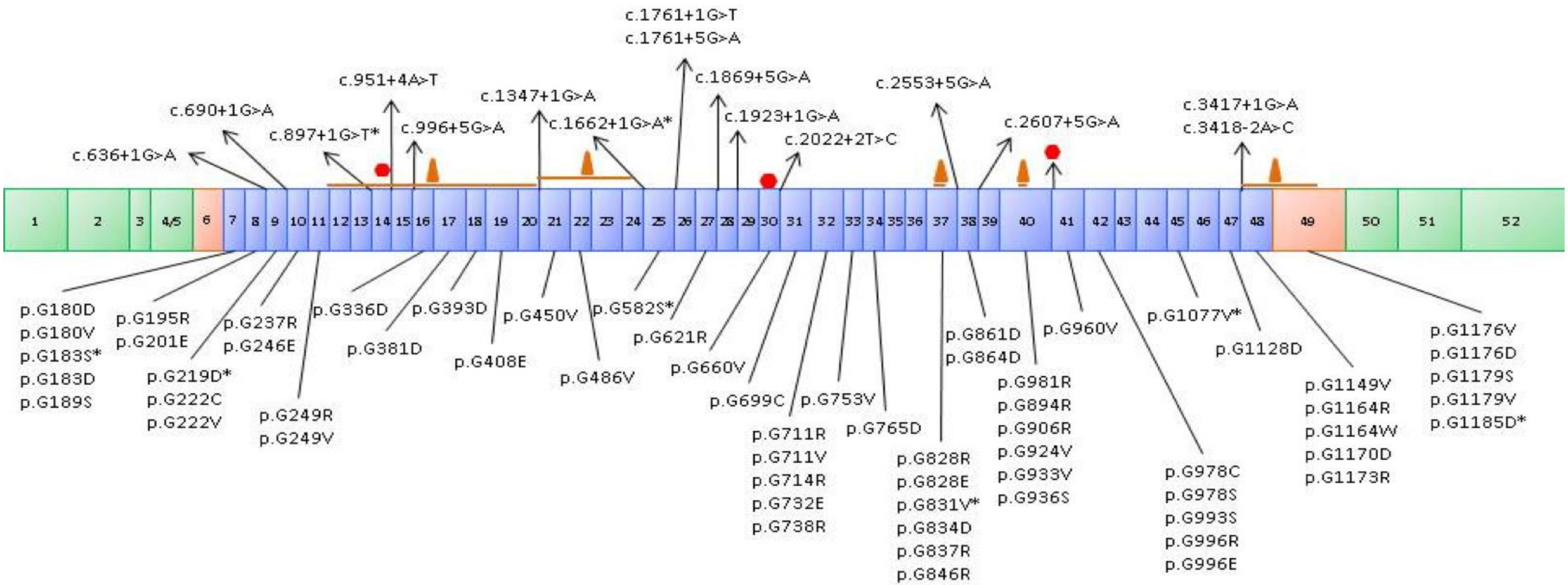
Type II collagen (*COL2A1*)



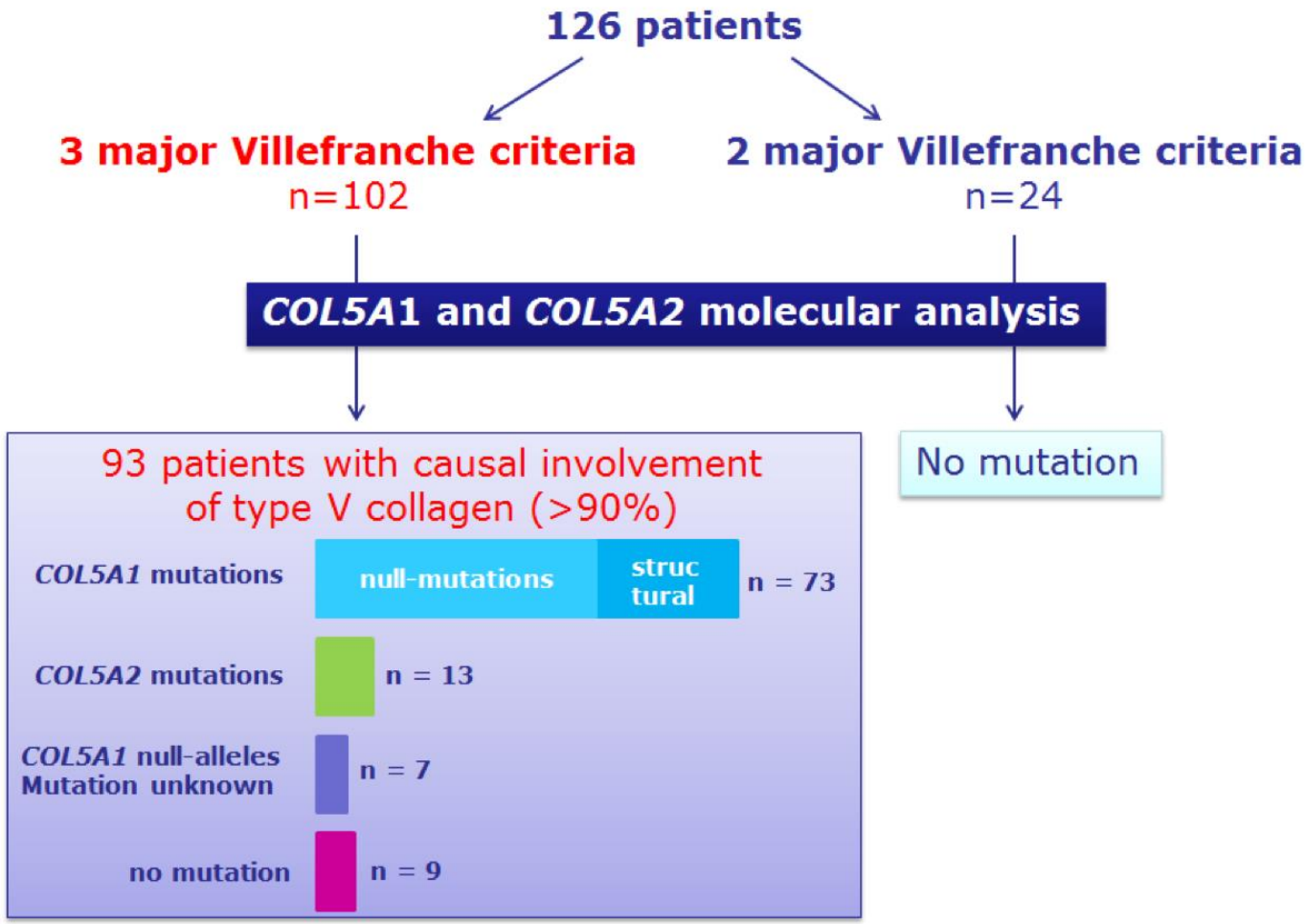
Vascular EDS (*COL3A1*)



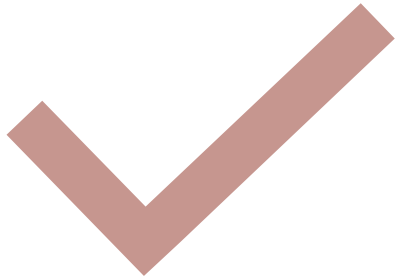
Vascular EDS (*COL3A1*)



Classic EDS (*COL5A1/COL5A2*)



Diagnostics in HCTD



Previously

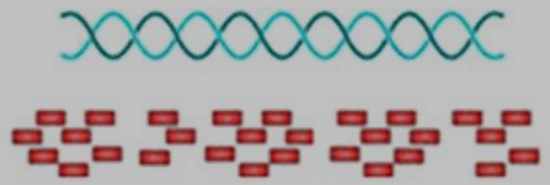
Gene-by-gene approach
Protein-driven

Current strategies

Genes simultaneous
"Gene"-driven

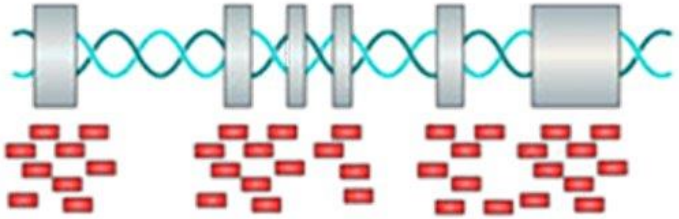
Current sequencing strategies

Whole genome sequencing



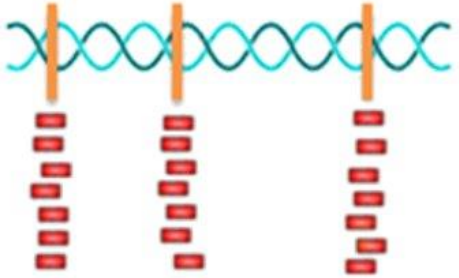
- Sequencing region : whole genome
- Sequencing Depth: >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

Whole exome sequencing



- Sequencing region: whole exome
- Sequencing Depth : >50X ~ 100X
- Identify all kinds of variants including SNPs, INDELs and SV in coding region.
- Cost effective

Targeted sequencing



- Sequencing region: specific regions (could be customized)
- Sequencing Depth : >500X
- Identify all kinds of variants including SNPs, INDELs in specific regions
- Most Cost effective

Targeted sequencing

"(Small)" gene (panels)

- Achondroplasia (*FGFR3*)
- ATS (2 genes)
- Craniosynostosis/Apert syndrome (*FGFR2*)
- Hypochondroplasia (*FGFR3*)
- Marfan syndrome
- Some single gene indications, e.g. Beals syndrome, achondrogenesis type 2, Brugada syndrome, Busschke-Ollendorff syndrome, …
- …

"Moderate" gene panels

- OI
- EDS
- FTAA
- Stickler syndrome
- HCMP – DCMP
- PXE
- Cutis laxa
- BM/UCMD
- …

Targeted sequencing

"(Small)" gene (panels)

Full coverage

- Achondroplasia (*FGFR3*)
- ATS (2 genes)
- Craniosynostosis/Apert syndrome (*FGFR2*)
- Hypochondroplasia (*FGFR3*)
- Marfan syndrome
- Some single gene indications, e.g. Beals syndrome, achondrogenesis type 2, Brugada syndrome, Busschke-Ollendorff syndrome, …
- …

Sanger sequencing - NGS

No TNXB in EDS gene panel

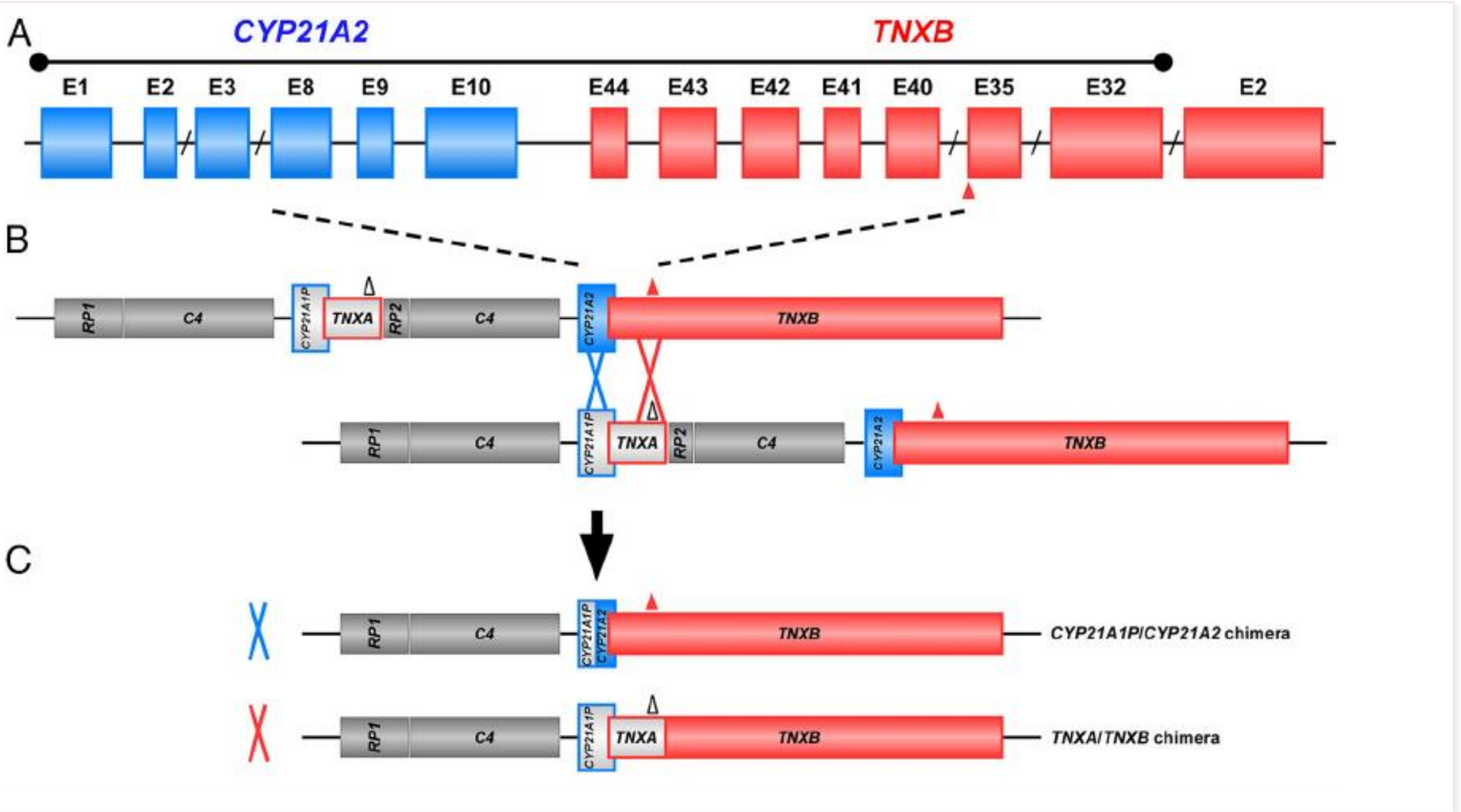
Targeted sequencing

"Moderate" gene panels

Full coverage

SEQCAP - HYPERCAP

- OI/Osteoporose/Hypophosphatasia: *ACAN, ALPL, B3GALT6, BMP1, COL1A1, COL1A2, CREB3L1, CRTAP, FAM46A, FKBP10, IFITM5, P3H1, LRP5, LRP6, MBTPS2, NBAS, P4HB, PLOD2, PLS3, PPIB, SEC24D, SERPINF1, SERPINH1, SP7, SPARC, TAPT1, WNT1, XYLT2, TMEM38B, LIFR, MESD, KDELR2, CCDC134, SGMS2, STX18, SUCO, WNT3A, PHEX, COPB2, ARF5, BICDL1*
- EDS: *ADAMTS2, AEBP1, B3GAT3, B4GALT7, C1R, C1S, CHST14, COL12A1, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, DSE, FKBP14, PLOD1, PRDM5, RIN2, SLC39A13, XYLT1, XYLT2, ZNF469, B3GALT6, FLNA, FLNB, TAB2*
- FTAA: *ACTA2, BGN, COL3A1, FBN1, FOXE3, HCN4, LOX, LTBP3, MAT2A, MFAP5, MYH11, MYLK, PRKG1, SMAD2, SMAD3, TGFB2, TGFB3, TGFBR1, TGFBR2, IPO8, EFEMP2*
- BM/UCMD: *COL12A1, COL6A1, COL6A2, COL6A3*
- HCMP: *ACTC1, CSPR3, JPH2, MYBPC3, MYH7, MYL2, MYL3, TNNC1, TNNI3, TNNT2, TPM1, PRKGA2*
- DCMP: *LMNA, TTN, RBM20, TNNC1, DES, TNNT2, FLNC, BAG3, LMNA, MYH7, SCN5A, TTN, DSP, NEXN, ACTC1, TPM1, JPH2, TNNI3, VCL*
- CCA/Beals: *FBN2*
- ATS: *SLC2A10, EFEMP2*
- Ectodermale dysplasie: *TP63, AXIN2, WNT10A, PAX9, MSX1, EDA, EDAR, EDARADD, GREM2, LRP6, WNT10B*
- Cutis laxa: *ELN, EFEMP2, FBLN5, LTBP4, ATP6V02, PYCR1, ALDH18A1, ATP7A, COG7, TALDO1, GORAB, NAA10, RIN2, LTBP1, LOX, EFEMP1, ATP6V1A, ATP6V1E1*
- Ectopia lentis: *LTBP1, ADAMTSL4, FBN1*
- Long QT syndroom: *SCN5A, KCNH2, KCNQ1, CALM2, CALM1, CALM3, TRDN, CACNA1C*
- MED: *COL2A1, SCL26A2*
- PXE: *ABCC6, ENPP1, GGCX, VEGFA, CYP2U1*
- Stickler syndroom: *COL2A1, COL11A1, COL11A2, COL9A1, COL9A2, COL9A3, LOXL3, LRP2*
- Vasculaire mineralisatie syndroom: *ANKH, NT5E, ENPP1*
- Weill-Marchesani syndroom: *ADAMTS10, ADAMTS17, FBN1, LTBP2, SMAD4*
- CPVT: *CASQ2, RYR2, TRDN, TECRL, CALM1, CALM2, CALM3*
- BAV: *GATA5, NOTCH1, NKX2-5, SMAD6, ROBO4*
- SED: *NKX3-2, COL2A1*


A
CYP21A2
TNXB
E1
E2
E3
E8
E9
E10
E44
E43
E42
E41
E40
E35
E32
E2
B
RP1
C4
CYP21A1P
TNXA
RP2
C4
CYP21A2
TNXB
RP1
C4
CYP21A1P
TNXA
RP2
C4
CYP21A2
TNXB
C
RP1
C4
CYP21A1P
CYP21A2
TNXB
CYP21A1P/CYP21A2 chimera
RP1
C4
CYP21A1P
TNXA
TNXB
TNXA/TNXB chimera

Targeted sequencing

"Moderate" gene panels

SEQCAP – HYPERCAP

THINGS TO KEEP IN MIND

- OI
- EDS
- FTAA
- Stickler
- HCMP – DCMP
- **PXE**
- Cutis laxa
- BM/UCMD
- …

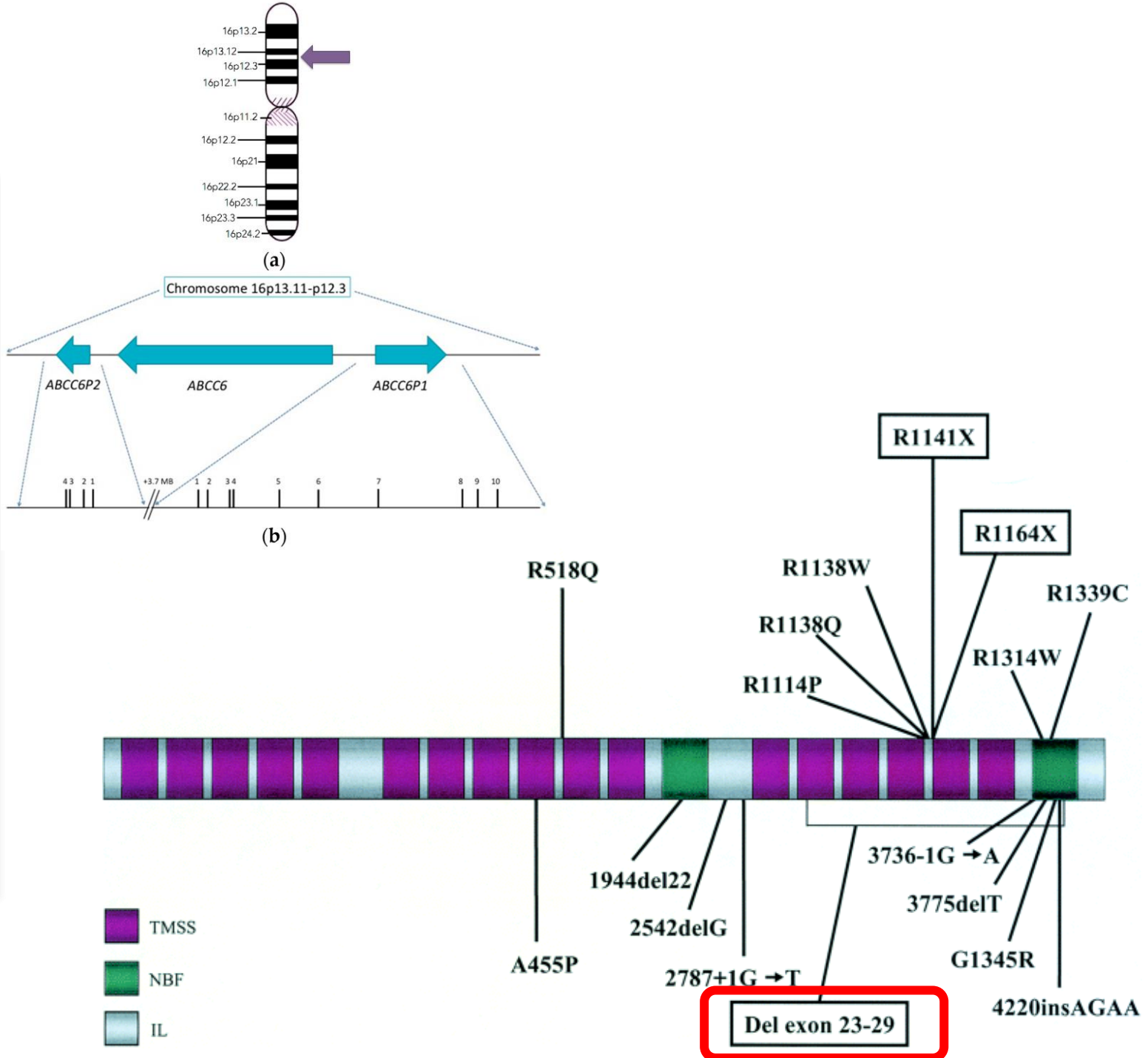
Targeted sequencing

"Moderate" gene panels

PXE – *ABCC6*

Caucasian population
approx. 15% del exon23-29

MLPA needed!



Targeted sequencing

"Moderate" gene panels

SEQCAP – HYPERCAP

THINGS TO KEEP IN MIND

- OI
- EDS
- FTAA
- Stickler
- HCMP – DCMP
- PXE
- Cutis laxa
- BM/UCMD

--> CNV detection needed

However: for most HCTD no commercial MLPA kit available! Solution: in-house design (software Exome Depth, MRC Holland design specific MLPA for each gene) --> limitation: positive controls needed, not available for all genes.

Whole exome sequencing

"Large" gene panels

= "dedicated" gene panels, linked to group of disorders

CNV detection, *in silico*

Trio analysis preferred

No full coverage

- Skeletal dysplasia
- Connective tissue panel: TNXB included, but: disclaimer!
- Skin disease (eg. FECH c.315-48T>C)
- …

Targeted sequencing/WES

Targeted sequencing

- For disorders with known disease genes/certainty clinical diagnosis.
- HyperCap: yearly re-evaluation of gene content. If needed: new design + optimization/validation.
- Relatively limited number of variants to interpret (dependent on gene content).
- Full coverage.
- Turnaround time is relatively short.

WES-based gene panels

- For group of disorders (eg skeletal dysplasia).
- Same commercial kit for all patients.
- Easy to extend the gene panel content.
- Interpretation of variants is more complex.
- No full coverage of all genes.
- Longer turn-around-time.
- Depending on the gene content: risk of incidental findings.

Questions?