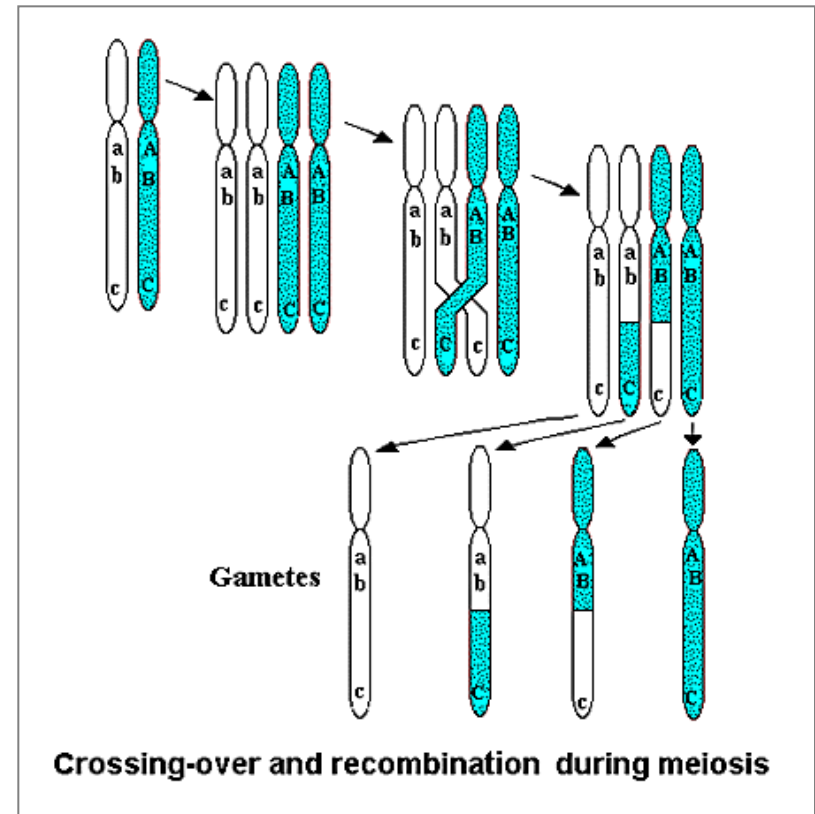
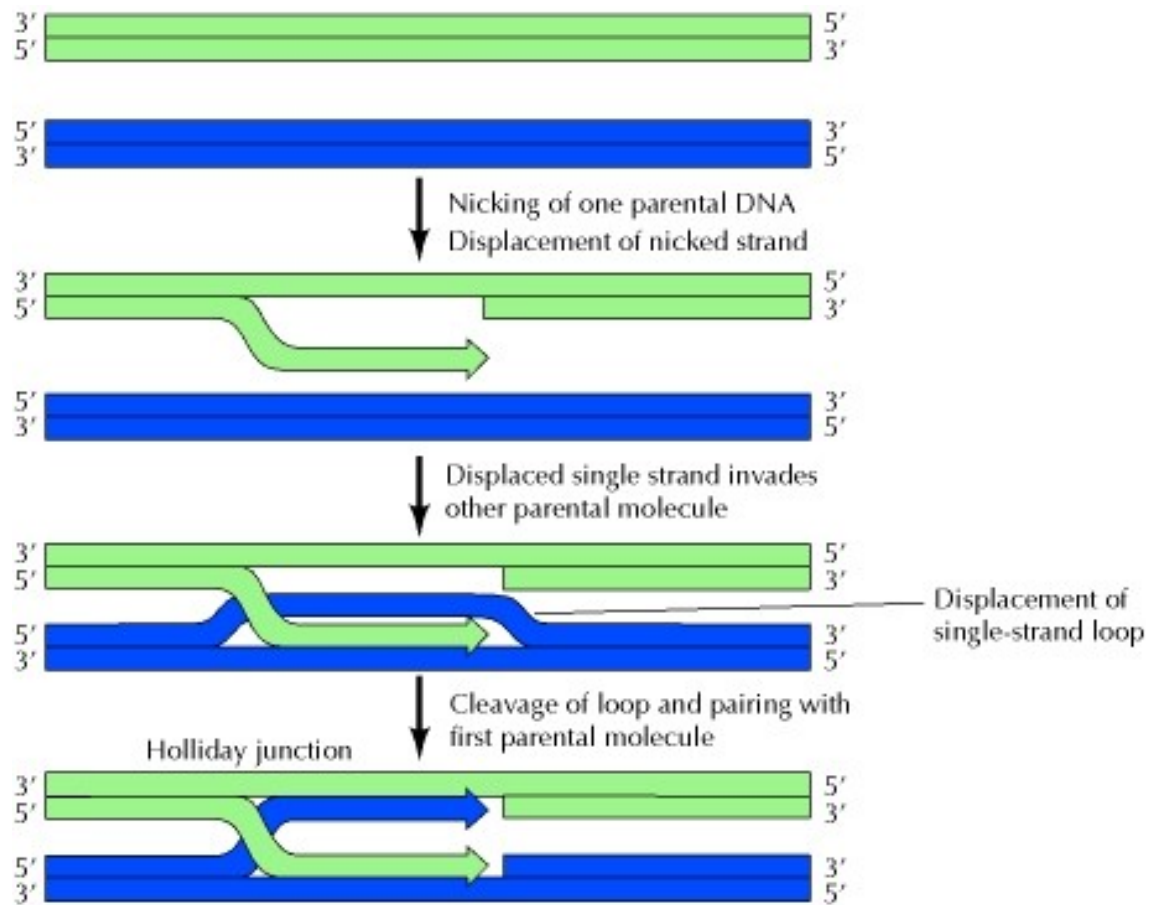
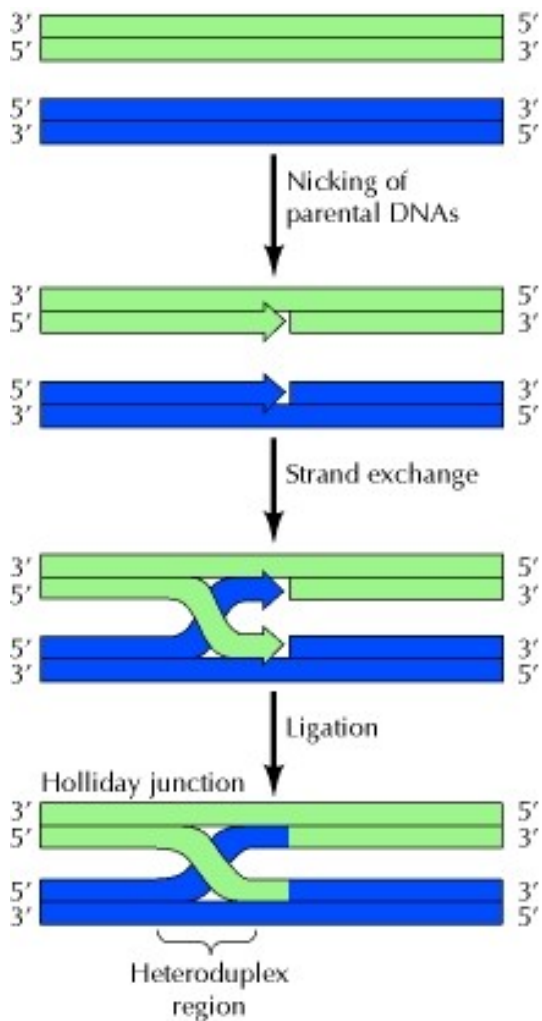


- DNA replikace a oprava DNA jsou základními kroky k uchování genetické informace a zabezpečení přenosu genetické informace.
- Geny na témže chromozomu by se měly dědit společně (jsou součástí téže struktury) – genová vazba.
- Genová vazba není absolutní – geny na témže chromozomu se mohou v průběhu meiózy od sebe oddělit a mohou se vytvořit nové kombinace genů – **rekombinace** → **evoluce a oprava DNA**.

- Při výměně se dva homologické chromozomy překříží.
- Místo překřížení – **chiasma**.
- U každé z chromatid dojde v místě překřížení ke zlomu a části chromatid se znovu spojí tak, že vytvoří rekombinanty.
- K rekombinaci dochází v profázi prvního meiotického dělení.
- Mnoho současných modelů **crossing-overu** bylo odvozeno od modelu, který v roce 1964 navrhl **Robin Holliday**.



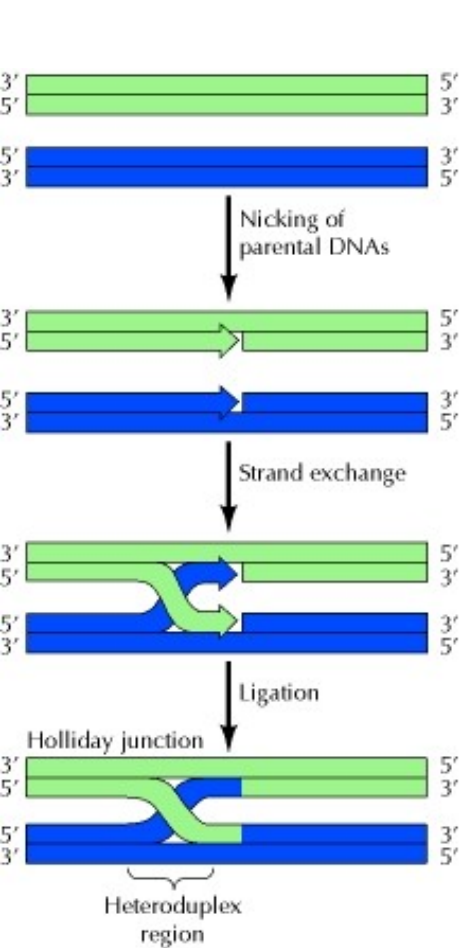


THE HOLLIDAY MODEL FOR HOMOLOGOUS RECOMBINATION

Single-strand nicks are introduced at the same position on both parental molecules. The nicked strands then exchange by complementary base pairing, and ligation produces a crossed-strand intermediate called a Holliday junction.

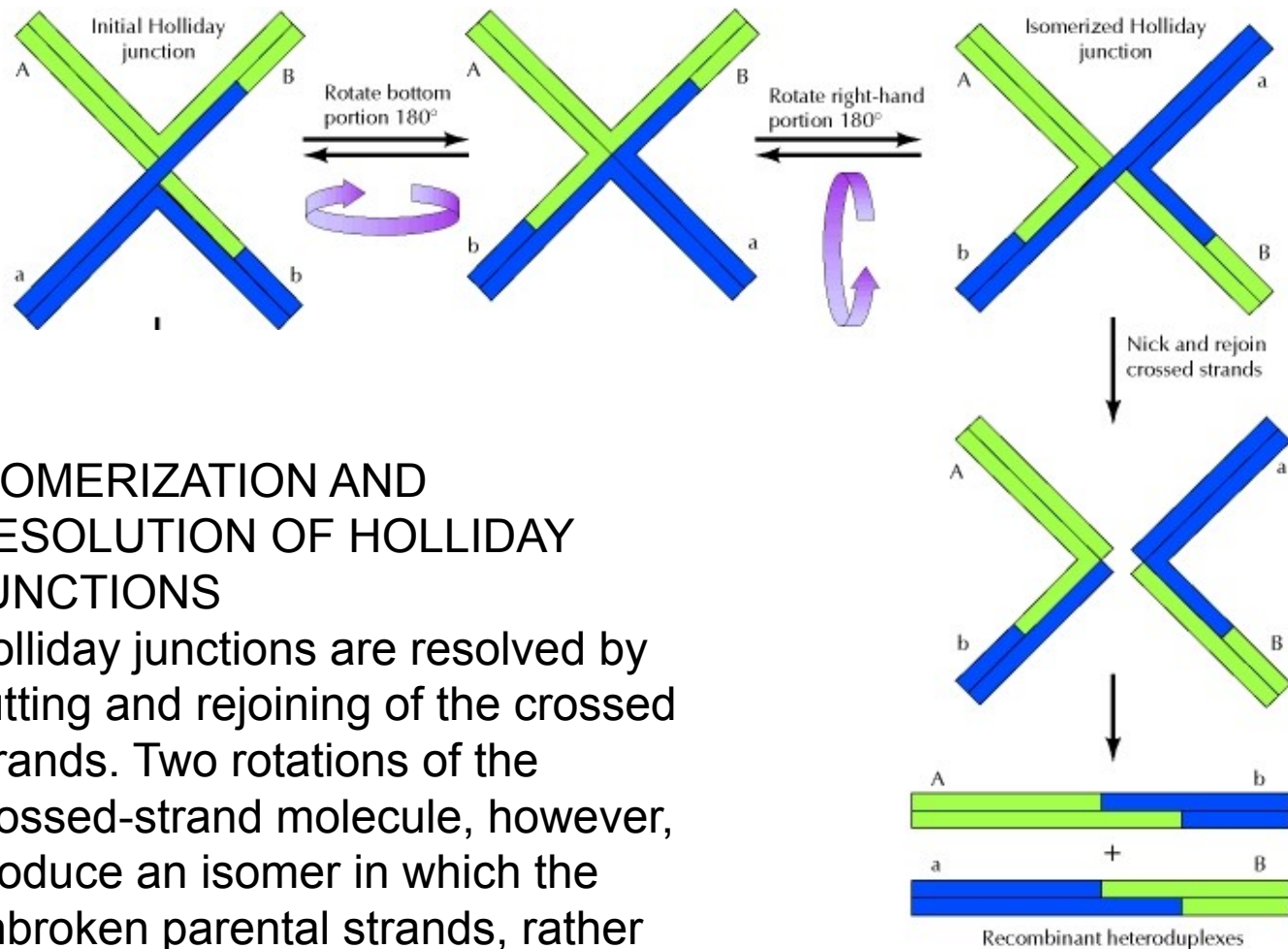
INITIATION OF RECOMBINATION BY NICKING ONLY ONE PARENTAL MOLECULE

The nicked DNA strand invades the other parental molecule by homologous base pairing, thereby displacing a single-stranded loop of DNA. This loop is then cleaved and pairs with the first parental molecule, yielding a crossed-strand Holliday junction.



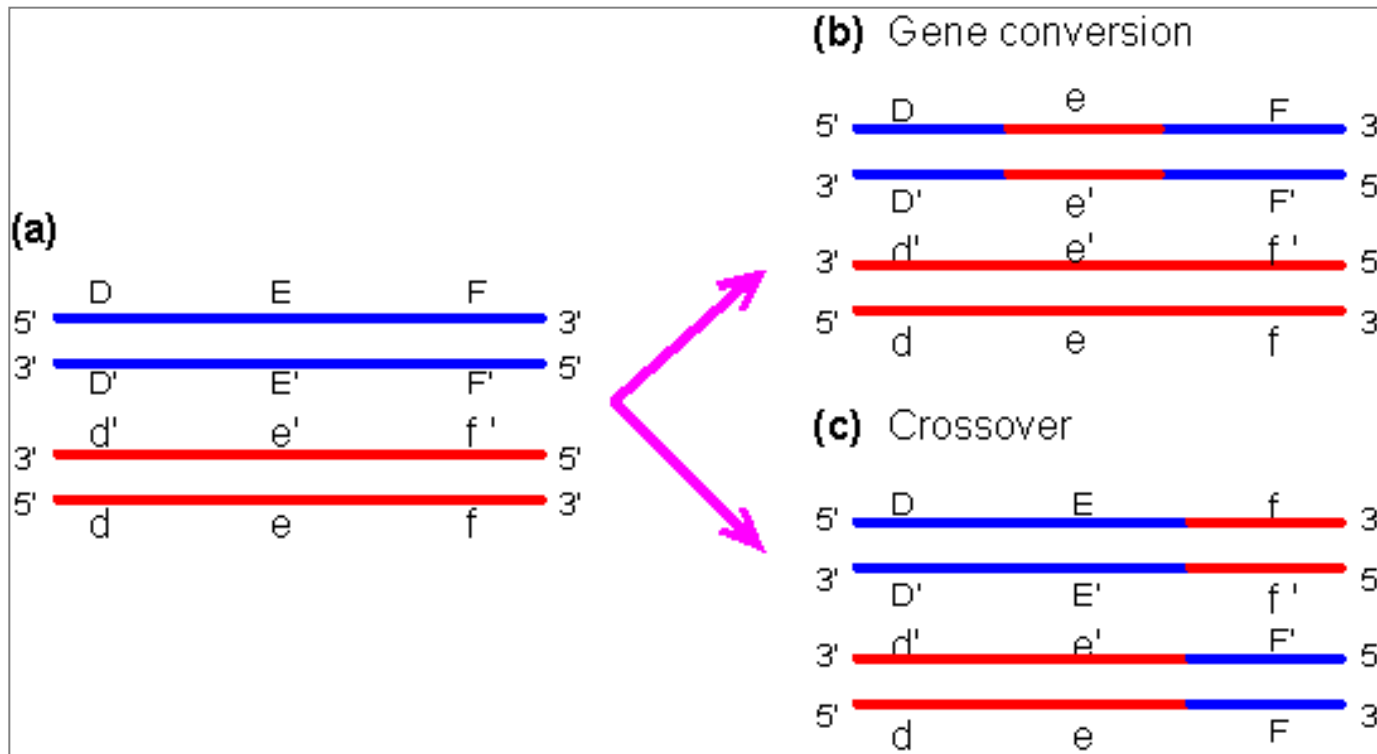
ISOMERIZATION AND RESOLUTION OF HOLLIDAY JUNCTIONS

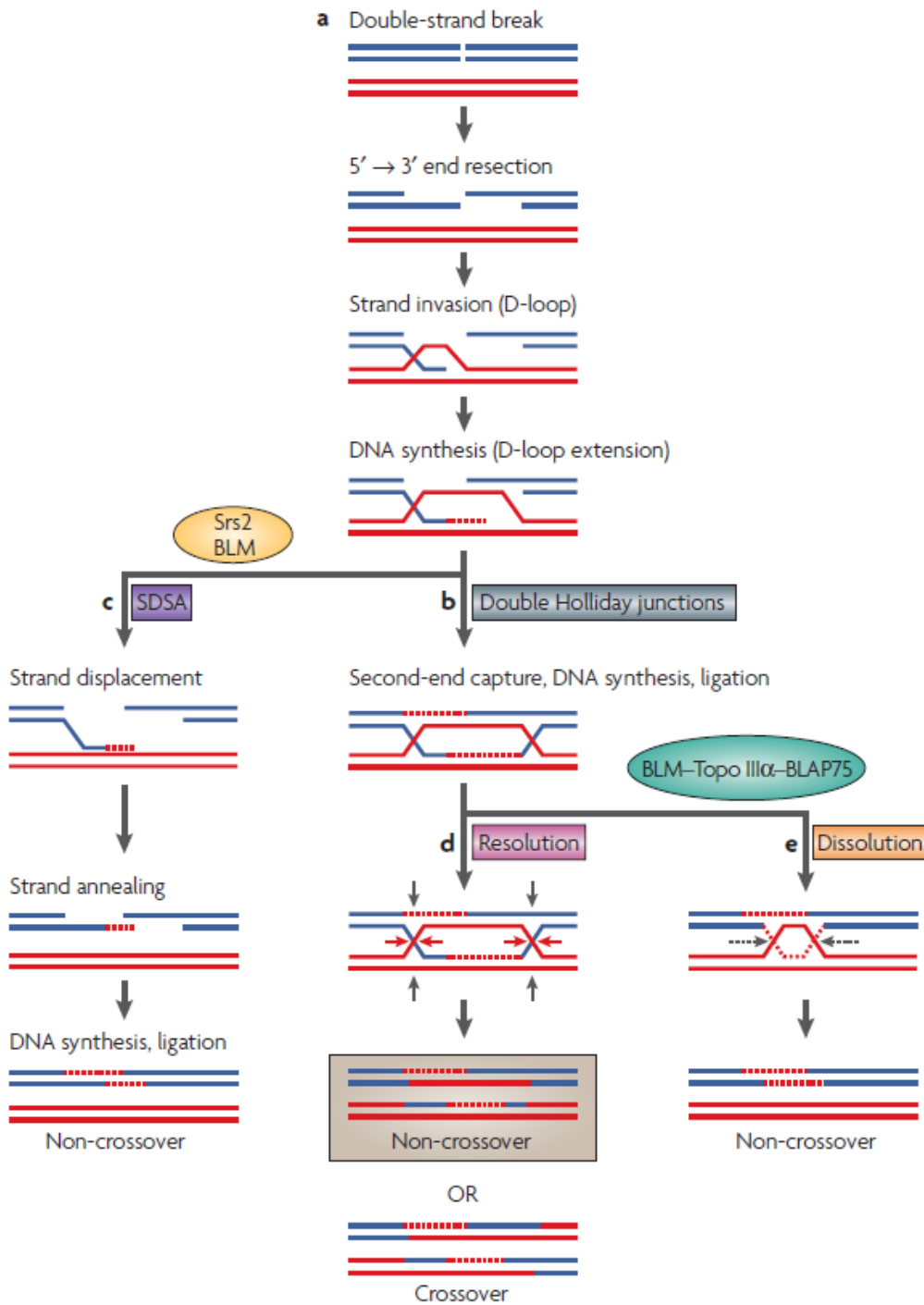
Holliday junctions are resolved by cutting and rejoining of the crossed strands. Two rotations of the crossed-strand molecule, however, produce an isomer in which the unbroken parental strands, rather than the initially nicked strands, are crossed. Cutting and rejoining of the crossed strands of this isomer yield progeny that are recombinant heteroduplexes.



Gene conversion is an event in DNA genetic recombination. It is a process by which DNA sequence information is transferred from one DNA helix (which remains unchanged) to another DNA helix, whose sequence is altered.

- Efficient gene conversion generally requires homology between interacting sequences, the homology between the interacting sequences is always >92% and usually >95%.

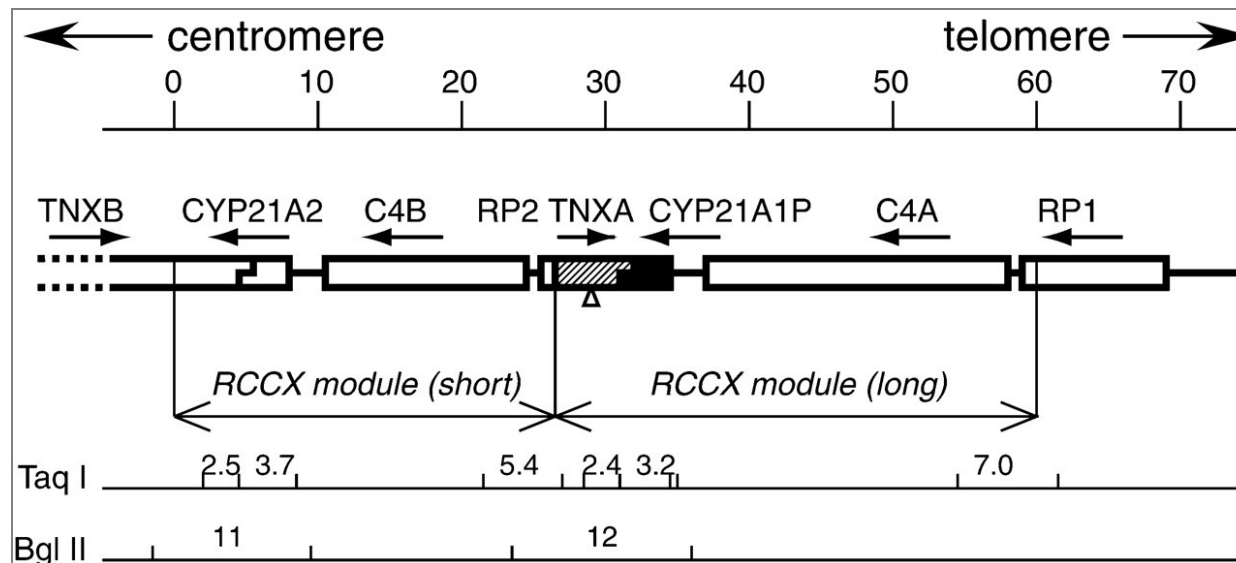




- The double-strand break (DSB); 3' ssDNA tails; the tails actively 'scan' the genome for homologous sequences, and one tail invades the homologous DNA duplex forming a D-loop, which is then extended by DNA synthesis.
- Synthesis-dependent strand annealing (SDSA) model: the invading strand and the newly synthesized DNA are displaced from the template and anneal to the other 3' end of the DSB, leading to the formation of **only gene-conversion events**.
- The other 3' end of the DSB is captured, and DNA synthesis and ligation of nicks lead to the formation of double HJs. The Bloom syndrome protein (BLM), topoisomerase III α and BLM-associated protein BLAP75 act together to remove the double HJs via convergent branch migration (indicated by dotted arrows at both HJs) **leading exclusively to gene conversion**. In DSBR, the resolution of the double HJs by an HJ resolvase is predicted to generate **an equal number of non-crossover and crossover events**.

nature reviews | genetics volume 8 | october 2007

- **Gene-conversion events have been implicated as the molecular cause of an increasing number of human inherited diseases.**
- Pathogenic gene conversion - **the transfer of genetic information from non-functional pseudogenes to their closely related functional genes.**
- Events in which pseudogenes have acted as donors resulted in the functional loss of the acceptor genes through the introduction of frameshifting, aberrant splicing, nonsense mutations, deleterious missense mutations and so on.



Overview of the *C4/CYP21* area, showing two RCCX modules as found on most chromosomes. *TNXB* is the full-size 68 kb gene for tenascin-X, *TNXA* (shown as a hatched box; also known as *XA*) is a truncated pseudogene of 5.7 kb that lacks most of the coding sequence of *TNXB* and has a deletion of 120 bp (indicated by the small triangle) spanning an exon–intron boundary. *CYP21A2* (also known as *CYP21B*) is the active steroid 21-hydroxylase gene; *CYP21A1P* (shown in black; also known as *CYP21A*) is a full-size pseudogene containing several deleterious mutations throughout its sequence, including three in-phase stop codons. The *C4* genes express variants of the fourth component of complement with different affinities, known as C4A and C4B. The arrangement with C4A in the telomeric and C4B in the centromeric module is common, but the specificity of the *C4* genes cannot be determined by means of the restriction sites shown, and many alternative arrangements have been described in the literature. About three-quarters of all RCCX modules are ‘long’ (33 kb in size); the others are ‘short’ (27 kb). The difference depends on the presence or absence of an endogenous retroviral sequence in one of the introns of the *C4* gene. The arrows show the orientation of transcription; there is an overlap between the 3′ sections of the oppositely transcribed genes *TNXB* and *CYP21A2*, and of *TNXA* and *CYP21A1P*, respectively. Bottom: characteristic *TaqI* and *BglII* restriction fragments. Top: scale in kb, with the centromeric RCCX duplication boundary at 0.

Hum. Mol. Genet. (2002) 11 (21): 2581-2590.

Table 1 | Interlocus gene-conversion events that cause human inherited disease* (part 1)

Disease/phenotype	Donor gene [‡]	Acceptor gene	Chromosomal localization	Directionality [§]	Mutation	Converted tract length (bp)
Atypical haemolytic uraemic syndrome	<i>CFHR1</i> ^{§§}	<i>CFH</i>	1q32	3'>5'	c.[3572C>T;3590T>C] [¶]	19–331
Congenital adrenal hyperplasia	<i>CYP21A1P</i>	<i>CYP21A2</i>	6p21.3	5'>3'	[-209T>C;-198C>T;-189/-188insT]	21–155
					[-4C>T;92C>T;118T>C;138A>C]	142–523
					[1380T>A;1383T>A;1389T>A; IVS6+12_13AC>GT]	42–210
					[1688G>T;1767_1768insT] [¶]	80–202
Syndrome of corticosterone methyloxidase II deficiency	<i>CYP11B1</i> ^{§§}	<i>CYP11B2</i>	8q21–q22	3'>5'	Conversion of exons 3 and 4	446–626
Increased 18-hydroxycortisol production	<i>CYP11B1</i> ^{§§}	<i>CYP11B2</i>	8q21–q22	3'>5'	Conversion of two nucleotides separated by two bases in exon 8	4–56
Autosomal dominant cataract	<i>CRYBP1</i>	<i>CRYBB2</i>	22q11.2–q12.1	3'>5'	c.[475C>T;483C>T]	9–104
Neural tube defects	<i>FOLR1P</i>	<i>FOLR1</i>	11q13.3–q14.1	5'>3'	7497_7662 including 13 discriminant nucleotides [¶]	166–215
					[7539T>C;7541G>A] [¶]	3–65
Gaucher disease	<i>GBAP</i>	<i>GBA</i>	1q21	3'>5'	[del55bp;D409H;L444P;A456P;V460V]	604–974
					[D409H;L444P;A456P;V460V]	525–848
					[L444P;A456P]	36–175
					[L444P;A456P;V460V]	50–475
Short stature	<i>GH2</i> ^{§§}	<i>GH1</i>	17q22–q24	3'>5'	Conversion involving 12 discriminant nucleotides in the promoter region	40–218
Mild microcytosis	<i>HBB</i> ^{§§}	<i>HBD</i>	11p15.5	3'>5'	Conversion involving exons 1 and 2	≥212–≤348 [¶]
Hereditary persistence of fetal haemoglobin	<i>HBG2</i> ^{§§}	<i>HBG1</i>	11p15.5	5'>3'	Conversion involving 9 discriminant nucleotides in the promoter region [¶]	423–1554
Agammaglobulinaemia	<i>IGLL3</i>	<i>IGLL1</i>	22q11.23	Inverted ^{**}	c.[393T>C;420T>C;425C>T]	33–152
Chronic granulomatous disease	<i>NCF1B</i> or <i>NCF1C</i>	<i>NCF1</i>	7q11.23	5' or inverted ^{**}	[C>T;ΔGT]	124–1474
					[C>T;ΔGT;ins20bp]	369–1529
					[ΔGT;ins20bp]	247–423
Blue cone monochromacy	<i>OPN1MW</i> ^{§§}	<i>OPN1LW</i>	Xq28	3'>5'	3' limit of the maximal converted region cannot be defined owing to the lack of information on an intervening discriminant nucleotide	≥636–≤3676 [¶]
Autosomal dominant polycystic kidney disease	? ^{††}	<i>PKD1</i>	16p13.3	? ^{††}	[8446T>G;8490T>C;8493G>C;8502T>C]	57–126
					[8446T>G;8490T>C;8493G>C;8498C>G;8502T>C]	57–126
					[8639G>T;8651G>A;8658T>C;8662C>T]	24–230
Chronic pancreatitis	<i>PRSS2</i> ^{§§}	<i>PRSS1</i>	7q35	3'>5'	Conversion involving 22 discriminant nucleotides	289–457

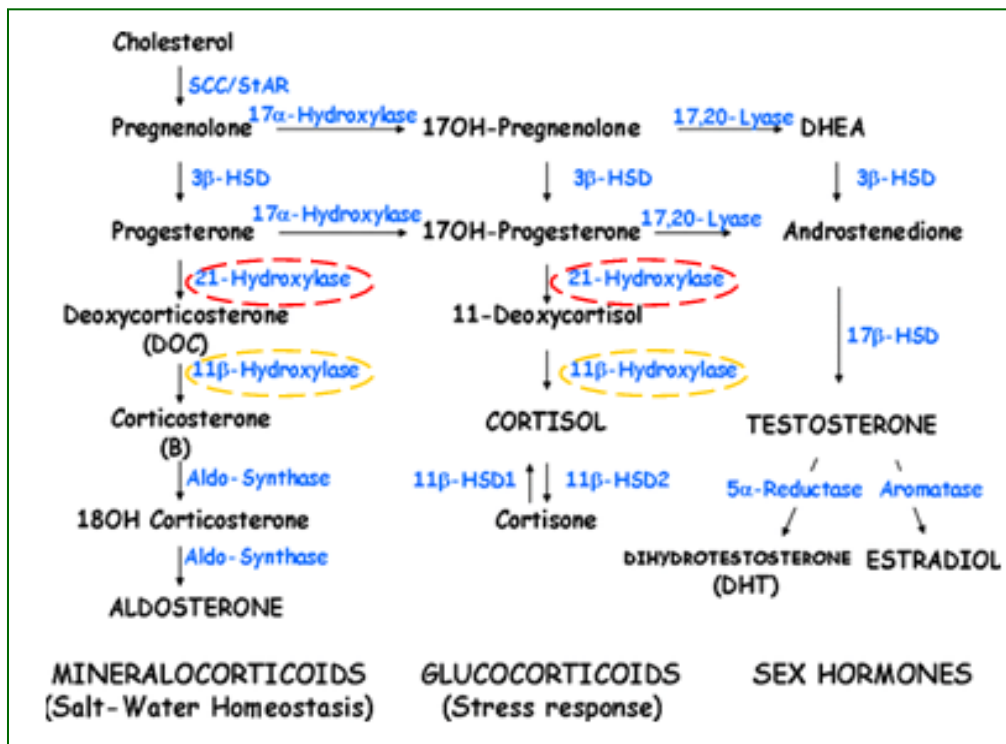


Table 1 | Interlocus gene-conversion events that cause human inherited disease* (part 2)

Disease/phenotype	Donor gene [‡]	Acceptor gene	Chromosomal localization	Directionality [§]	Mutation	Converted tract length (bp)
Shwachman-Bodian-Diamond syndrome	<i>SBDSP</i>	<i>SBDS</i>	7q11.22	Inverted**	c.[129-443A>G;129-433G>A]	11-104
					c.[141C>T;183_184TA>CT]	44-217
					c.[141C>T;183_184TA>CT;201A>G]	61-276
					c.[141C>T;183_184TA>CT;201A>G;258+2T>C] [¶]	120-398
					c.[183_184TA>CT;201A>G]	19-118
					c.[183_184TA>CT;201A>G;258+2T>C]	78-240
					c.[201A>G;258+2T>C]	60-197
Spinal muscular atrophy	<i>SMN2</i>	<i>SMN1</i>	5q13.2	5'>3'	Conversion of exon 7 and intron 7	264-776
von Willebrand disease	<i>VWFP</i>	<i>VWF</i>	22q11.22-q11.23 (<i>VWFP</i>)/12p13.3 (<i>VWF</i>)	Not applicable	[IVS27-45C>T;IVS27-36C>T;3686T>G;3692A>C]	63-131
					c.[3686T>G;3692A>C]	7-95
					c.[3686T>G;3692A>C;3735G>A;3789G>A;3797C>T]	112-195
					c.[3789G>A;3797C>T]	9-99
					c.[3789G>A;3797C>T;3835G>A]	47-195
					c.[3789G>A;3797C>T;3835G>A;3931C>T;3951C>T]	163-291
					c.[3835G>A;3931C>T;3951C>T]	117-229
					c.[3835G>A;3931C>T;3951C>T;4027A>G;4079T>C;4105T>A]	271-335
					c.[3931C>T;3951C>T]	21-191
c.[3931C>T;3951C>T;4027A>G;4079T>C;4105T>A]	175-297					

Kongenitální adrenální hyperplasie (CAH)

- zahrnuje autosomálně recesivní enzymové defekty steroidogeneze v kůře nadledvin s různým biochemickým a klinickým obrazem



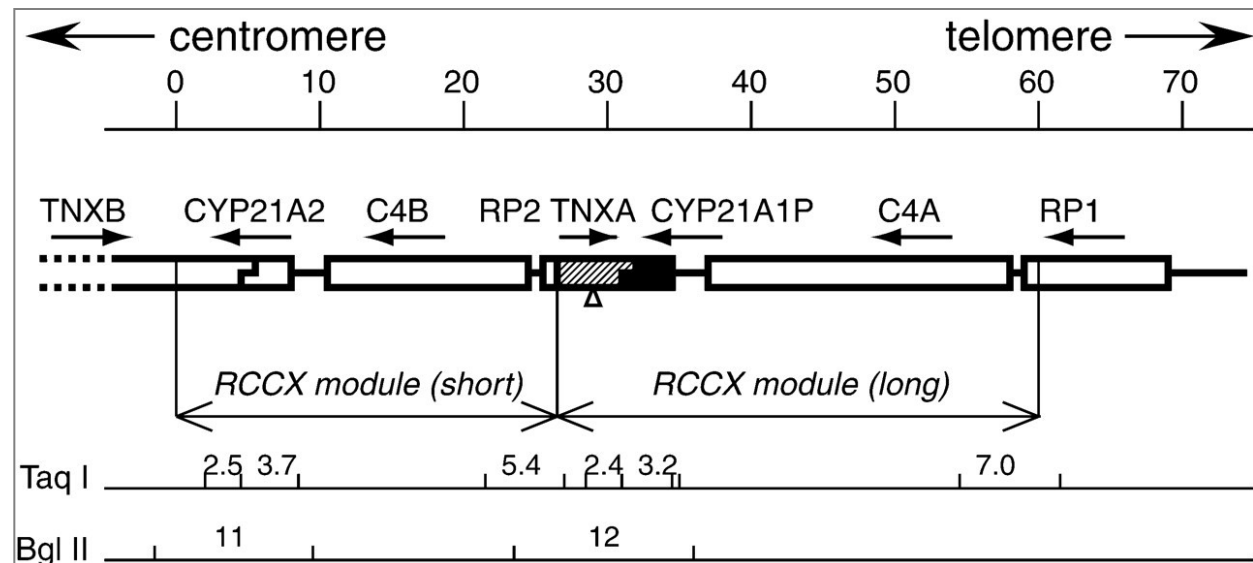
- DNA diagnostika CAH - **analýza genu pro 21-hydroxylázu** (zavedena od roku 2003)

- Novorozenecký screening deficitu 21-hydroxylázy** (zaveden od roku 2007)

Deficit 21-hydroxylázy (21-OHD)

- způsoben mutacemi v genu **CYP21A2**
- **CYP21A2** gen a **CYP21PA1P** pseudogen, lokalizace na chromozómu 6p21.3
- **CYP21A2** a **CYP21PA1P**: každý obsahuje 10 exonů, nukleotidové sekvence jsou v exonech identické z 98% a v intronech z 96%

- incidence:
v ČR 1: 11 000



Klinické formy 21-OHD

1. Klasická se solnou poruchou (Salt Wasting)

enzymová aktivita: 0% normální aktivity

nejčastější mutace: **p.Q318X**, **p.G110VfsX21**, **klastr E6**,
p.R356W, **chimérní gen a delece genu**

2. Prostá virilizující (Simple Virilizing)

enzymová aktivita: 1% normální aktivity

nejčastější mutace: **p.I172N** a **c.290-13A/C>G**

3. Neklasická (Non Classic-late onset)

enzymová aktivita: 20-50% normální aktivity

nejčastější mutace: **p.P30L** a **p.V281L**

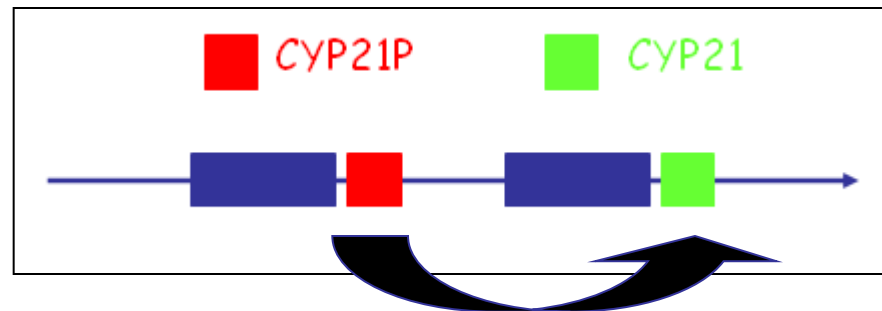
3 clinical forms of CAH have been distinguished:

- **the classical salt-wasting (SW-CAH),**
 - **the classical simple virilising (SV-CAH),**
 - **the non-classical CAH (NC-CAH).**
- In addition to having a severe cortisol deficiency, patients with SW-CAH do not synthesise enough aldosterone and therefore, are not able to maintain sodium homeostasis. In the female foetus, the excess of androgen causes variable degrees of external genital virilisation, and consequently, newborn females have genital ambiguity.
- The residual activity of 21OH (aldosterone is synthesized) results in SV-CAH with external genital virilisation in females and also results in signs of precocious pseudopuberty which develop by 8 years of age in female and male patients.
- NC-CAH is associated with a moderate deficiency in 21OH and manifests in later childhood or adolescence with precocious pseudopuberty and decreased fertility. Patients with NC-CAH secrete aldosterone normally and most of the male patients diagnosed after puberty are entirely asymptomatic

Mutace způsobující 21-OHD

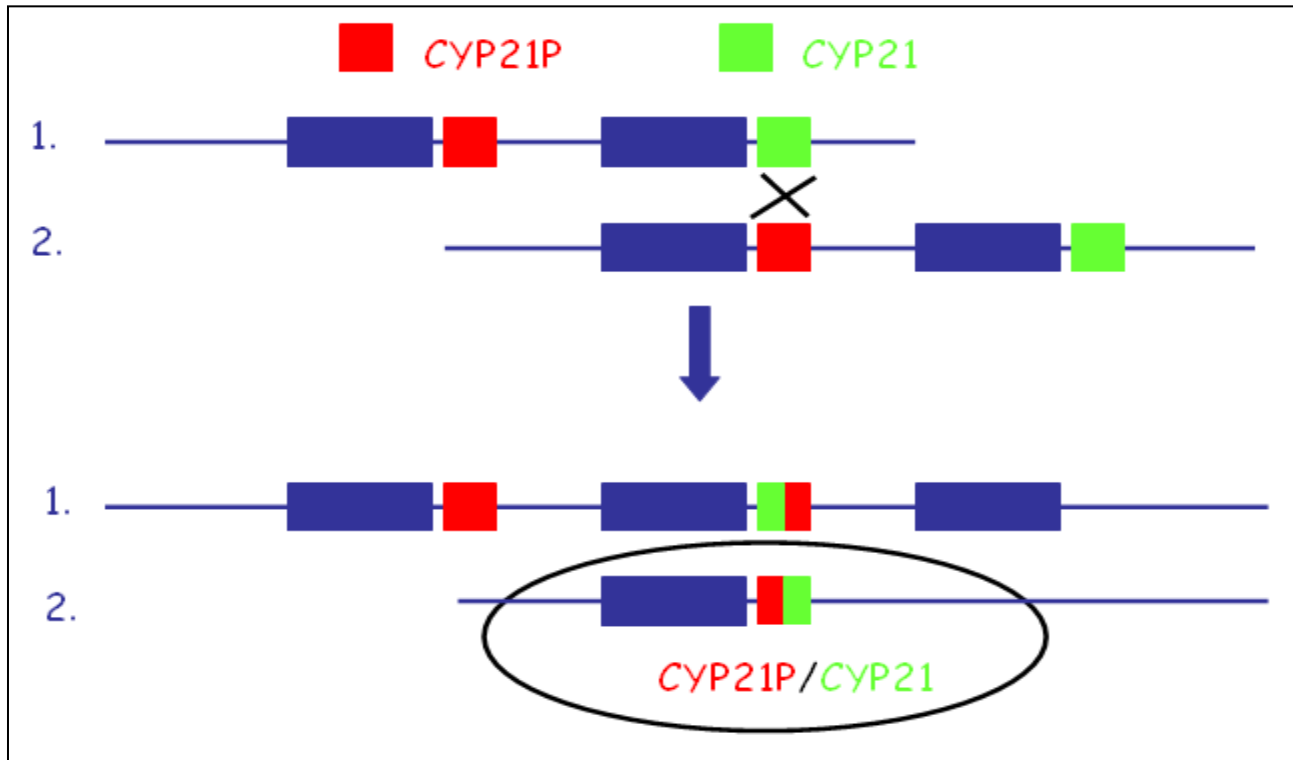
1/ intergenové rekombinace mezi CYP21 genem a CYP21P pseudogenem ~ 95%

→ mikrokonverze
· bodové mutace



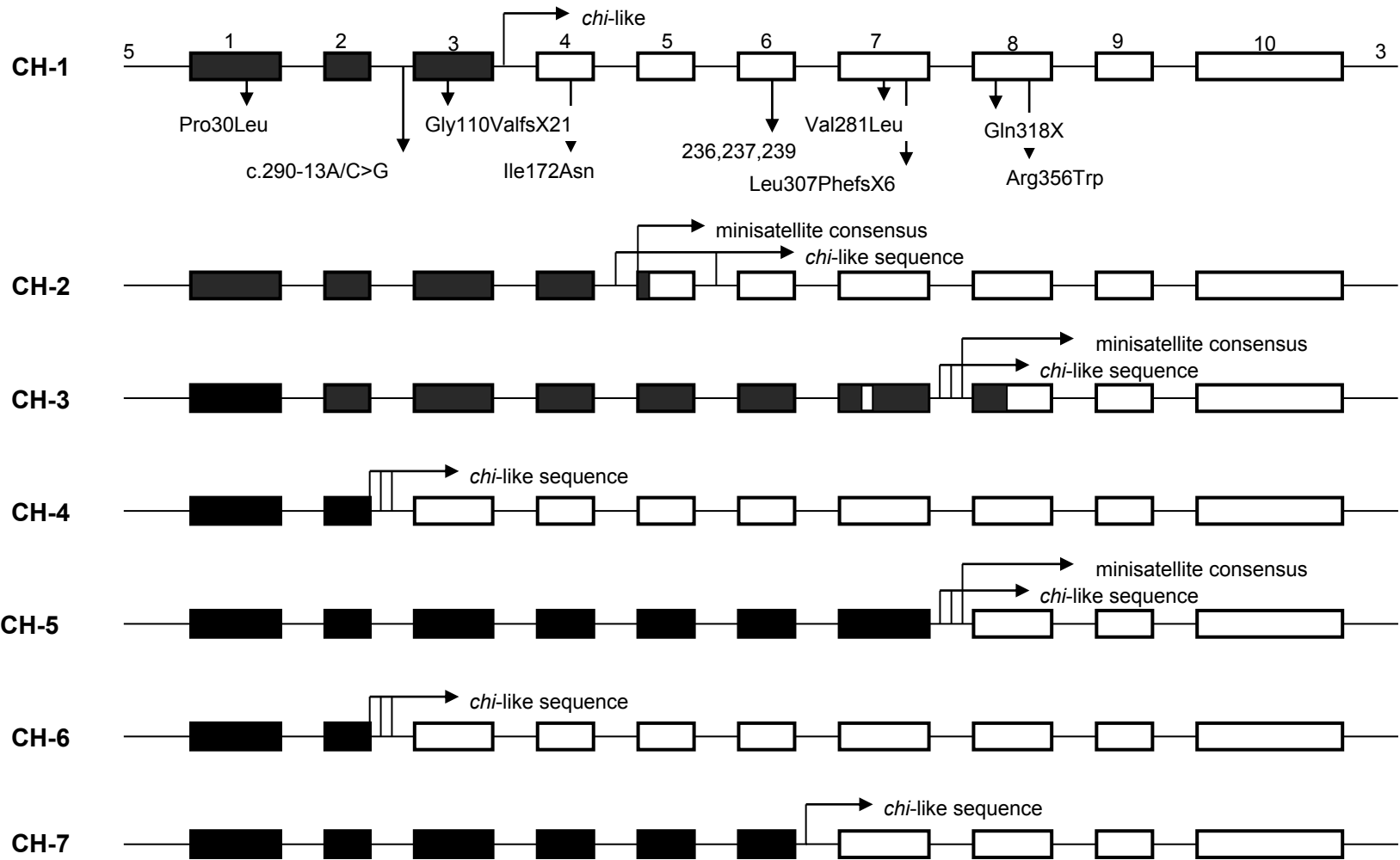
→ nerovnoměrný crossing-over během meiózy
· chimérní CYP21P/CYP21 gen
· delece CYP21 genu
· duplikace CYP21 genu

Vznik chimérického CYP21P/CYP21 genu pomocí nerovnoměrného crossing-overu během meiózy



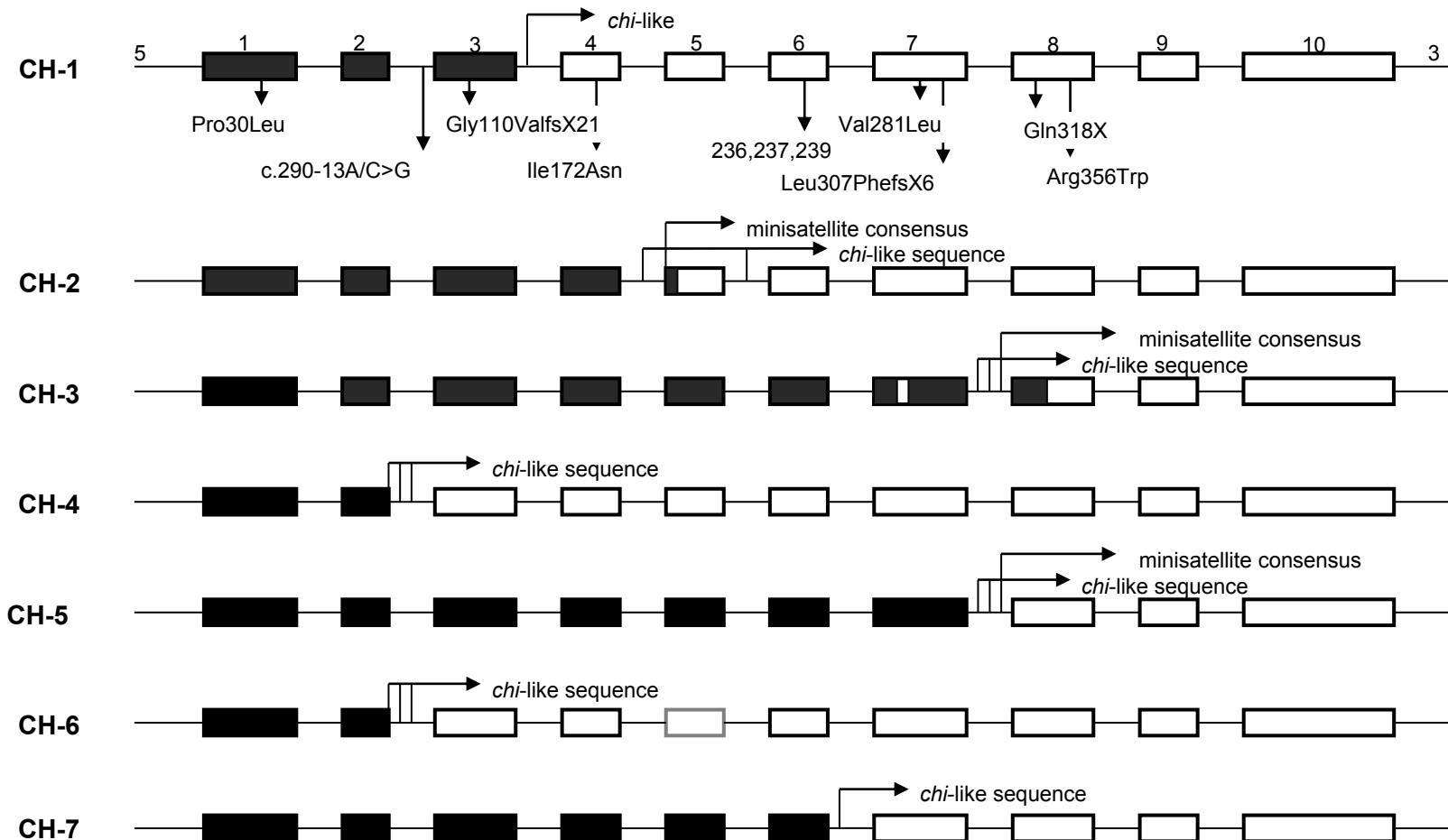
2/ mutace v CYP21 genu ~ 5%

- nově vzniklé bodové mutace

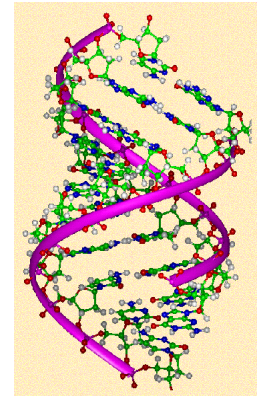


Types of chimeric *CYP21A1P/CYP21A2* genes. The structure of the functional *CYP21A2* gene is depicted by white boxes; black boxes represent the nonfunctional *CYP21A1P* pseudogene. The arrows indicate mutations which exist in *CYP21A1P*. 236/237/239 depicts mutations p.Ile236Asn, p.Val237Glu, and p.Met239Lys, these mutations always occur together. In CH-4, the *CYP21A1P-CYP21A2* junction site is localised inside intron 2 upstream of c.290-13A/C>G. In CH-6, the *CYP21A1P-CYP21A2* junction site is localised between mutations c.290-13A/C>G and p.Gly110ValfsX2.

- Specific motifs surrounding DNA sequences involved in gene conversion can either promote or inhibit gene-conversion. Such motifs include polypurine and polypyrimidine tracts, palindrome-like sequences, **minisatellite sequences**, ***chi* and *chi*-like sequences** and sequences that can adopt tetraplex, Z-DNA,
- *Chi* (crossover hotspot instigator) sites had been originally described as cis-acting motifs 5'-GCTGGTGG-3' around which the rate of Rec-promoted recombinations is elevated



Metodika 21-OHD



1. MLPA

- duplikace CYP21 genu
- delece CYP21 genu
- bodové mutace (p.G110VfsX21; p.I172N; klastr E6; p.Q318X)

2. genově selektivní Long Expand Template PCR

- amplifikace CYP21 genu
- amplifikace CYP21P/CYP21 chimérodního genu

3. sekundární PCR / ACRS

- průkaz 11 mutací: p.P30L, c.290-13A/C>G, p.G110VfsX21, p.I172N, klastr E6 (p.I236N, p.V237E, p.M239V), p.V281L, p.Q318X, p.R356W, L306FfsX6

4. PCR-sekvenování

Naše výsledky u 21-OHD

- ♣ DNA analýza provedena u 267 nepříbuzných pacientů:
 - diagnóza potvrzena u 241 probandů
 - u 26 pacientů identifikována pouze 1 mutantní alela
- ⇒ **charakterizováno celkem 30 typů mutantních alel**
- 60% bodové mutace - 58,6% z CYP21P (24 % c.290-13A/C>G)
 - 2,4% nově vzniklé mutace
- 33,7% chimérní CYP21P/CYP21 geny - 4 typy
- 4,9% delece CYP21 genu
- 1,0% CYP21 geny nesoucí 2 a více bodových mutací
- 0,4% duplikace CYP21 genu (c.290-13A/C>G; p.Q318X)

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 26: 595-603, 2010

Identification of *CYP21A2* mutant alleles in Czech patients with 21-hydroxylase deficiency

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Original article

Chimeric *CYP21A1P/CYP21A2* genes identified in Czech patients with congenital adrenal hyperplasia

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Kazuistika

deficitu 21-hydroxylázy

r. 2003

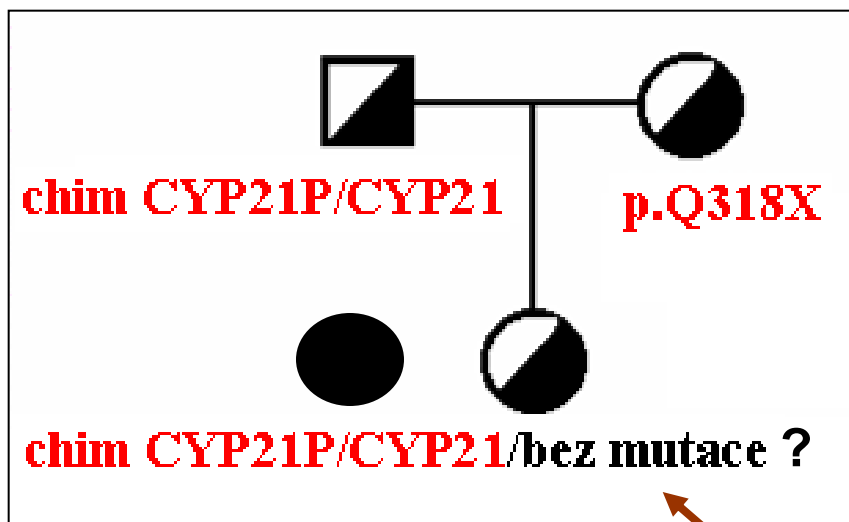
- žádost o DNA analýzu pro potvrzení diagnózy CAH u probandky a pro prenatální dg. (matka v 9. týdnu gravidity)
 - DNA byla dostupná od obou rodičů a postiženého dítěte: 7letá dívka, v 5 letech věku nástup předčasné puberty, středně virilizovaný zevní genitál, bazální 17-OHP > 133 nmol/l
- fenotyp: klasická forma bez solné poruchy

Výsledky DNA analýzy rodiny

1. genově selektivní LR PCR
2. PCR/ACRS - 10 mutací
3. LR PCR - delece genu

→ detekce bodové mutace **Q318X** v HT stavu
(8. exonu CYP21 genu)

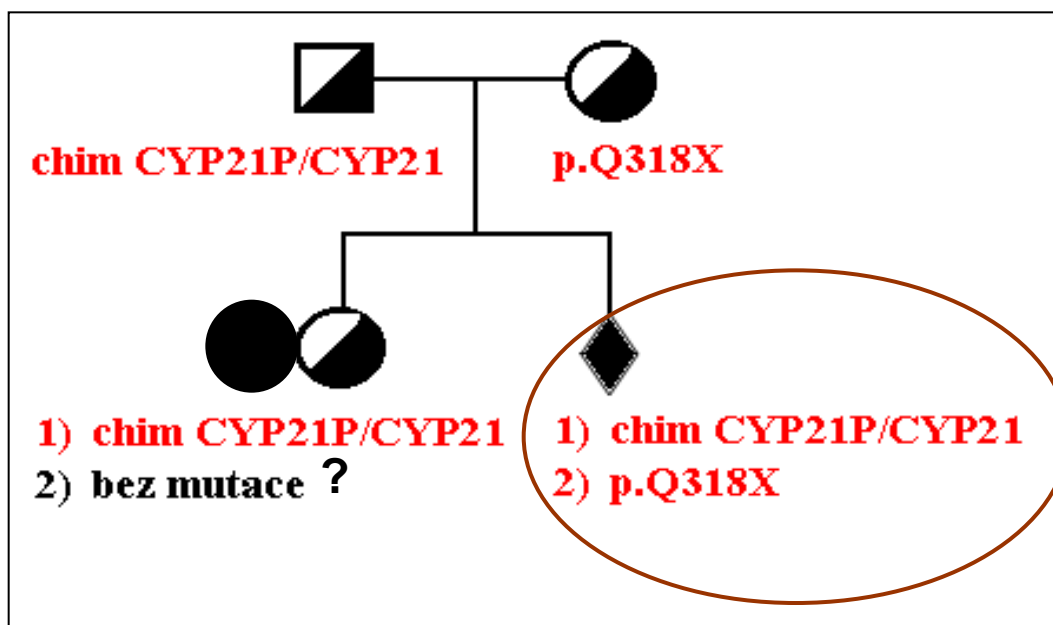
→ detekce **chimérodního CYP21P/CYP21 genu**
v HT stavu



PROČ NASTALA
NESHODA MEZI
GENOTYPEM A
FENOTYPEM ???

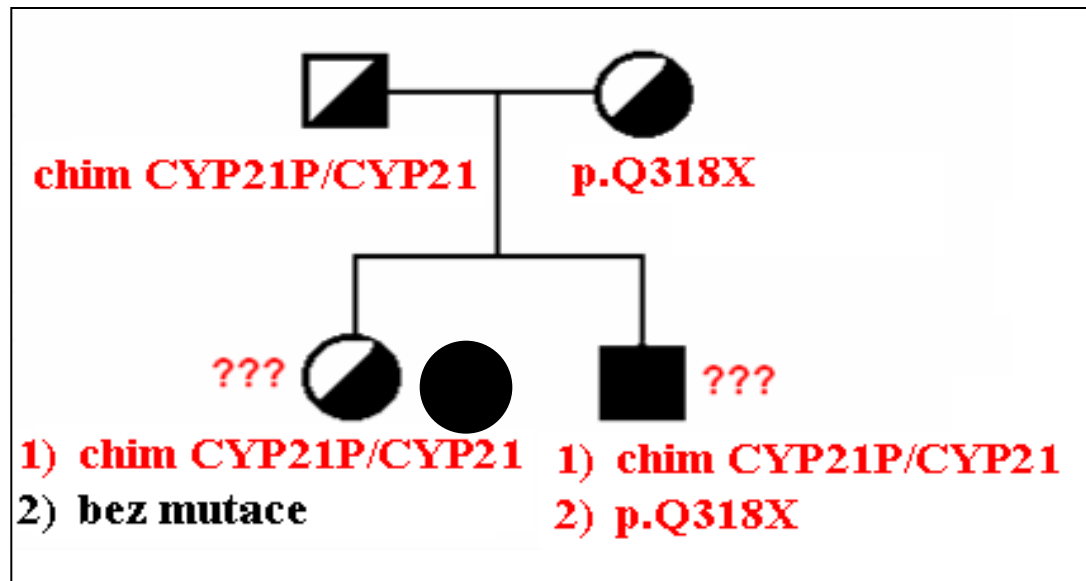
Rok 2003 - prenatální diagnostika

- kultivované AMC
- výsledný genotyp plodu odlišný od genotypu probandky



X biochemické stanovení plodové vody: negativní výsledek !!!
($C_{17-OHP}=4,81$ nmol/l; $N_{17-OHP}=3-17$ nmol/l)

Po narození syna bylo provedeno ověření genotypu z prenatální dg. - stejný výsledek, dítě však nemělo klinické projevy CAH !!!

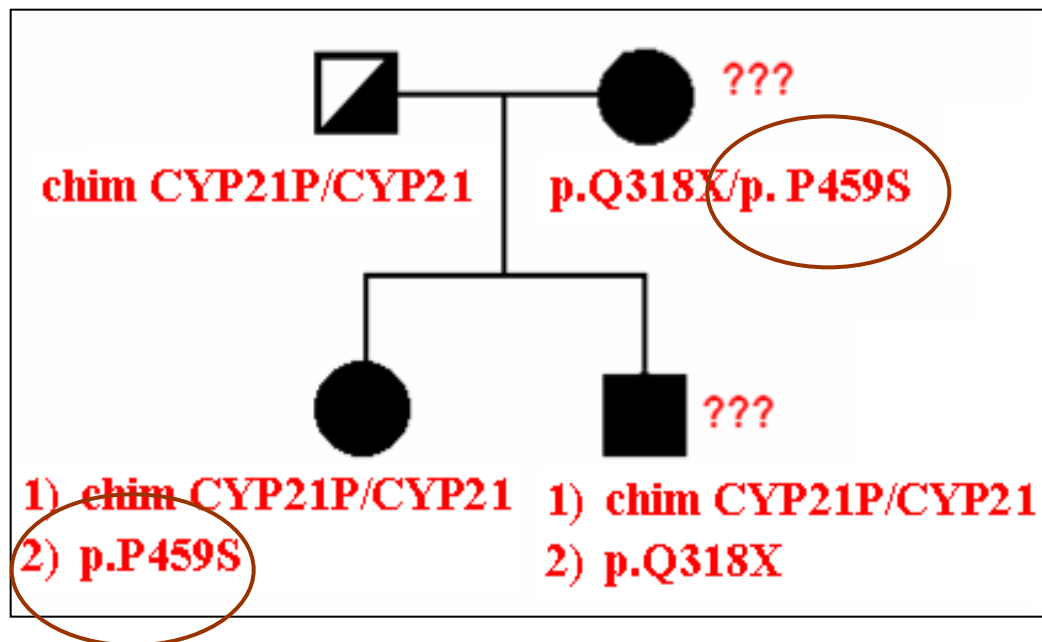


PROČ TRVÁ V RODINĚ NESHODA MEZI GENOTYPEM A FENOTYPEM ???

r. 2005 - zdokonalení DNA diagnostiky CAH:

1) sekvenování celé kódující sekvence

→ detekce bodové mutace p.P459S v HT stavu na maternální alele (10. exonu CYP21 genu)

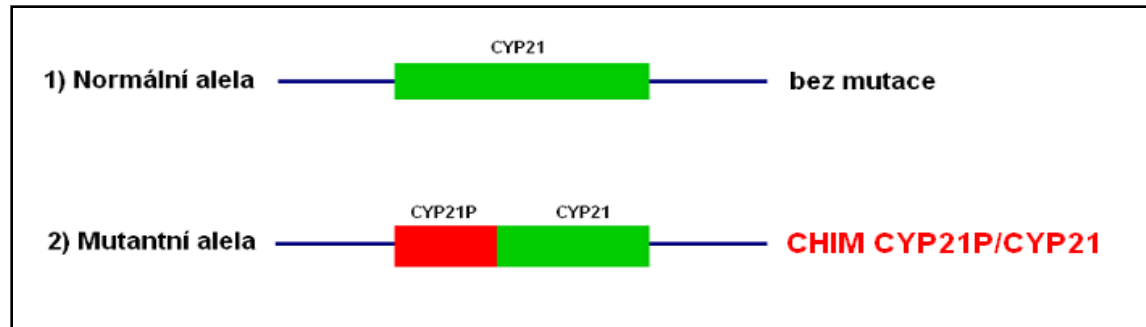


r. 2007 - zdokonalení DNA diagnostiky CAH:

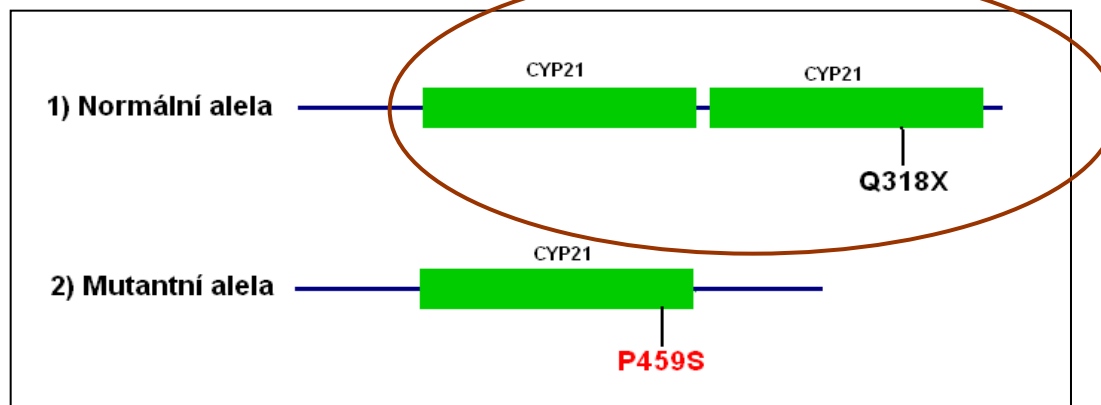
2) MLPA analýza přestaveb 6p21.3 oblasti

→ detekce duplikace CYP21 genu s p.Q318X v HT stavu na maternální alele

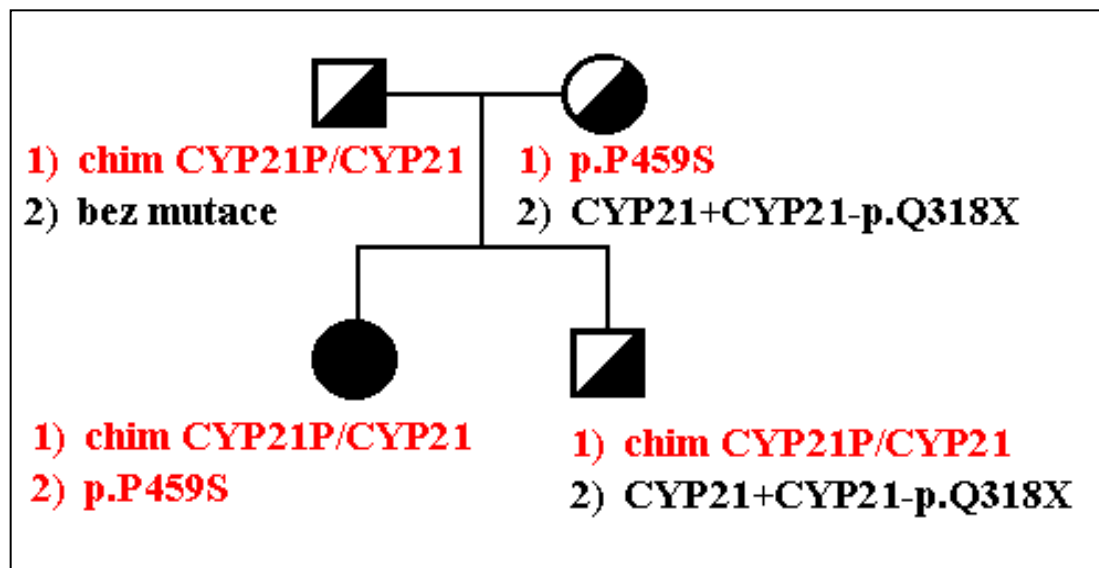
A/ Otec:



B/ Matka:



Analýza rodiny byla uzavřena s tímto konečným výsledkem:



Familial hypercholesterolemie (FH) -

mutace v genu pro **low-density lipoprotein receptor (LDLR)** -
frekvence heterozygotů (1:500), frekvence homozygotů (1:1000000)

Patogenní mutace v LDL receptoru způsobují **ztrátu funkčních receptorů pro LDL částice na povrchu jaterních buněk** → **zvýšení hladiny LDL v plazmě** → ateroskleróza a zvýšené riziko koronární srdeční nemoci



Fig. 1 - Xanthelasma - case 1.

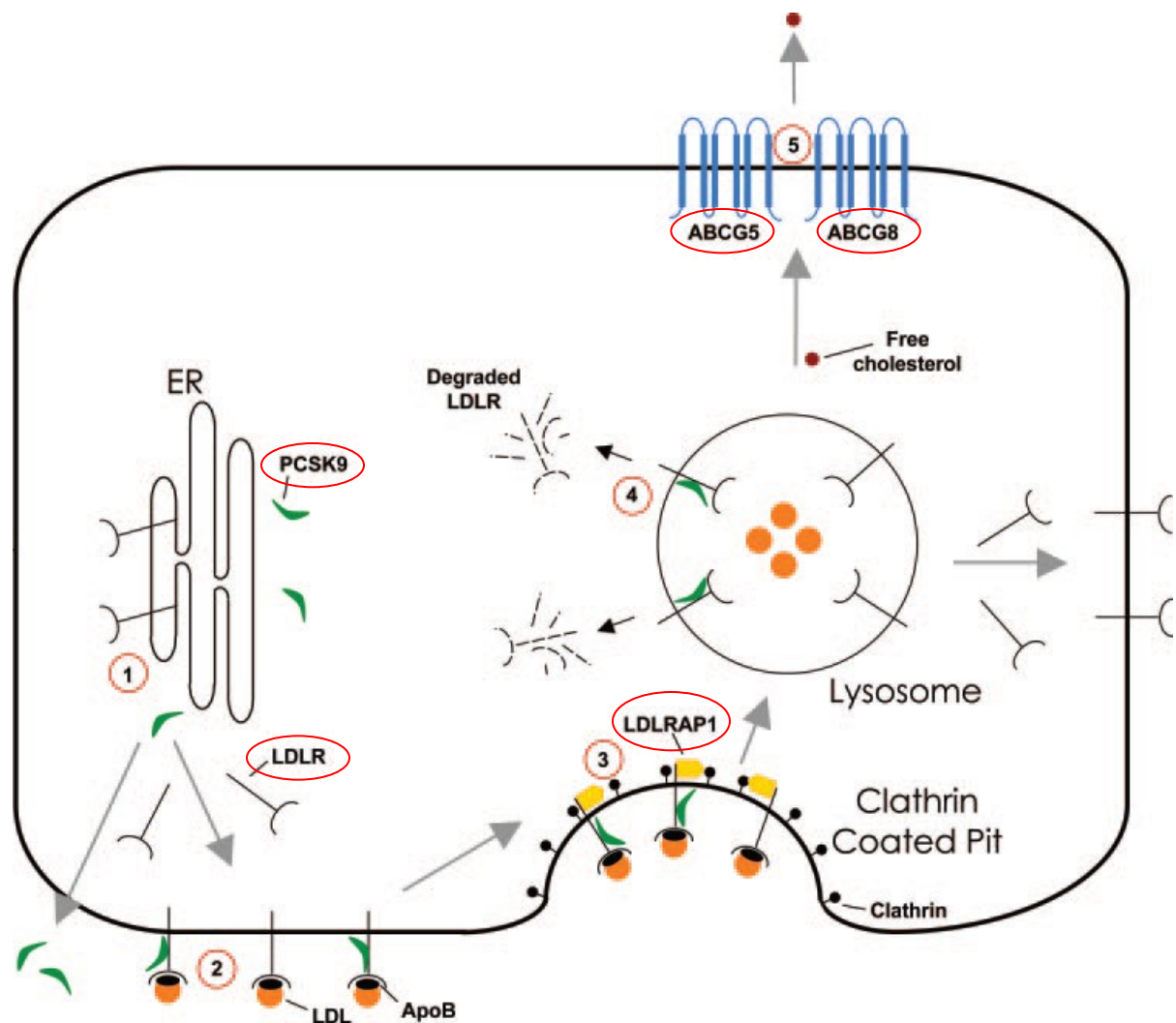


Fig. 5 - Tendinous xanthomas and xanthoma tuberosum in the elbow in case 2, prior to ileal bypass surgery.



Fig. 2 - Tendinous xanthomas in the fingers in case 1, prior to ileal bypass surgery.

V endoplazmatickém retikulu probíhají posttranslační úpravy **LDL receptoru**, který je následně transportován k buněčné membráně. Na buněčné membráně dohází prostřednictvím specifické interakce s **ApoB (apolipoprotein B)** k tvorbě komplexů LDLR-LDL částice. **PCSK9 (proprotein convertase subtilisin-like kexin type 9)** je sekretován z hepatocytů a na jejich povrchu se váže LDLR. LDLR (vázaný i nevázaný k PCSK9) je následně shromážděn v záhybech bohatých na kltrin a internalizován za pomoci **LDLRAP1 (LDLR adaptor protein)**. Po endocytóze jsou LDL částice disociovány z LDLR a degradovány v lysozomu. LDLR je recyklován zpátky k buněčné membráně nebo degradován (LDLR vázaný na PCSK9).



Mechnizmy mající za následek vznik FH:

- i) Defektní struktura a funkce LDL receptoru,
- ii) Defektní vazba ApoB/LDLR,
- iii) Defektní struktura a funkce LDLRAP1 (LDLR adaptor protein) (narušená endocytóza ApoB/LDLR),
- iv) Abnormálně zvýšená aktivita PCSK9 (zvýšený katabolismus LDLR),
- v) Defektní ABCG5/8 (zvýšení intrabuněčné akumulace cholesterolu v hepatocytech).

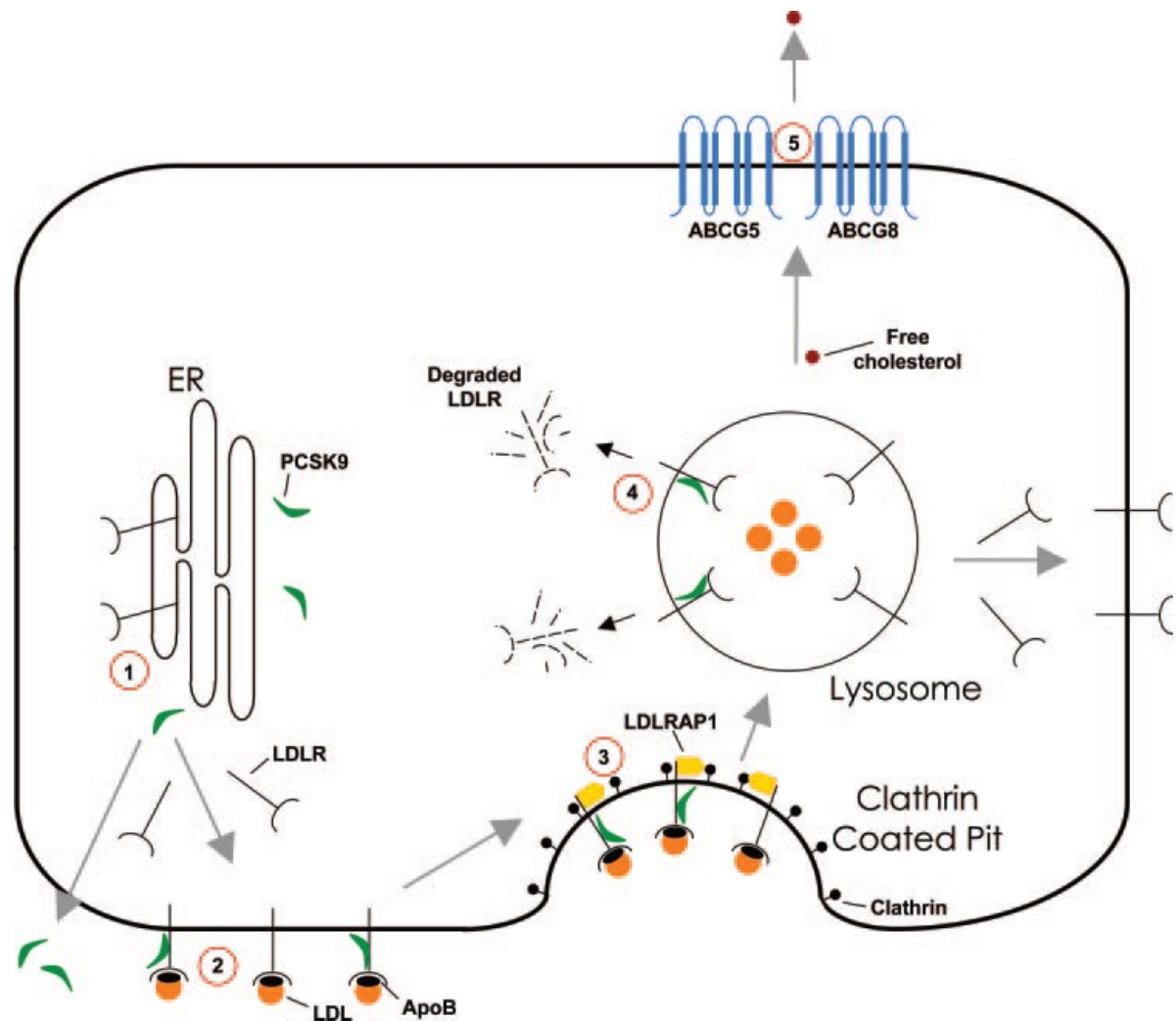
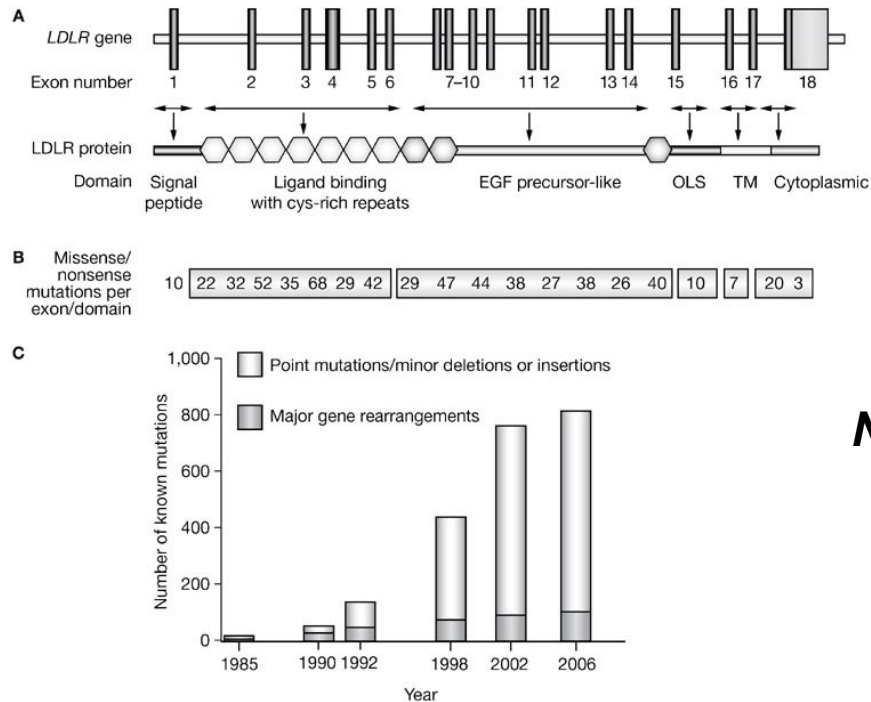


Figure 3 The *LDLR* gene



Number of unique allelic variants (2009): 1050

Soutar AK and Naoumova RP (2007) Mechanisms of Disease: genetic causes of familial hypercholesterolemia
Nat Clin Pract Cardiovasc Med 4: 214–225 doi:10.1038/ncpcardio0836

- The LDLR gene is localized at 19p13.2, is composed of 18 exons spanning 45 kb, the transcript is 5.3 kb long and encodes a peptide containing 860 amino acids.
- LDLR mutations have been reported along the whole length of the gene in FH patients from around the world.
- **At present, the number of identified unique LDLR allelic variants is over 1000: 65% of the variants are DNA substitutions, 24% small DNA rearrangements (< 100 bp) and 11% large DNA rearrangements (> 100 bp)**

Molekulární diagnostika FH

- 1) PCR/RFLP nejčastější mutace v genu *APOB* - p.Arg3527Gln
- 2) PCR/RFLP pro detekci nejčastějších mutací v genu *LDLR* - p.Gly592Glu, p.Asp266Glu, and p.Arg416Trp;
- 3) PCR a sekvenování exonu 4 genu *LDLR* (exon s největším výskytem mutací);
- 4) MLPA všech exonů genu *LDLR*;
- 5) PCR a sekvenování promotoru a exonů 1, 5, 6, 9, 10, 12, 14 genu *LDLR*;
- 6) DHPLC exonů 2, 3, 7, 8, 11, 13, 15, 16, 17, and 18 genu *LDLR*, sekvenování vytypovaných exonů

Výsledky: 1945 nepříbuzných pacientů s FH - 252 pacientů (13,0%) s *APOB* mutací, 406 pacientů (20,9%) s bodovou mutací nebo mutací malého rozsahu v *LDLR*, 37 pacientů (1,9%) s mutací velkého rozsahu v *LDLR*.

V současné době: genotypovací FH čip: detekce 80 typů mutací popsaných u českých FH pacientů a 77 mutací detekovaných s vysokou frekvencí v jiných evropských populacích + MLPA

Large DNA
rearrangements
detected in Czech FH
patients

<i>Deletion/duplication</i>	<i>No. of probands</i>	<i>No. of patients</i>
Promoter-ex1del	1	2
promoter_ex2del	1	3
Exon2_10del	1	1
Exon3_12del	1	1
Exon5_10del	4	8
Exon7_10del	1	4
Exon7_12del	1	1
Exon9_14del	11	18
Exon9_15del	9	19
Exon11_17del	1	1
Exon13_14del	1	1
Exon16_17del	1	3
Exon1_12dup	1	1
Exon2_6dup	14	18
Exon2_15dup	1	2
Exon4_8dup	2	5
Exon16_18dup	6	18

Genomic characteristics of deletion and duplication breakpoints in the LDLR gene in Czech FH patients

Mutation at cDNA level	Mutation at DNA level	Deletion/duplication size	Recombination mechanism	Repetitive element 5'/class/family	Repetitive element 3'/class/family	No. of probands
promoter_2exon del	c. -1823_190+566 del	13186bp	NAHR	AluY/SINE/Alu	AluY/SINE/Alu	1
exon2_6dup	c. 67+3968_940+296dup	14228bp	NAHR	AluSx1/SINE/Alu	AluSx3/SINE/Alu	9
exon3_12del	c.190+984_1846-1160del	17604bp	NAHR	FLAM_A/SINE/Alu	AluY/SINE/Alu	1
exon4_8dup	c.314-446_1187-386dup	8119bp	NHEJ	AluSx1/SINE/Alu	MER83/LTR/ERV1	1
exon5_10del	c. 695-67_1586+371del	7636bp	NHEJ	AluJo/SINE/Alu	AluSx1/SINE/Alu	4
exon9_14del)	c.1186+700_2141-545del	10291bp	NAHR	AluYa5/SINE/Alu	AluY/SINE/Alu	10
exon9_15del)	c.1187-169_2312-790del	14110bp	NAHR	AluJb/SINE/Alu	AluSx1/SINE/Alu	8
exon16_18dup)	c.2311+1941_*1216dup	7248bp	NAHR	AluYb8/SINE/Alu	AluSq2/SINE/Alu	3

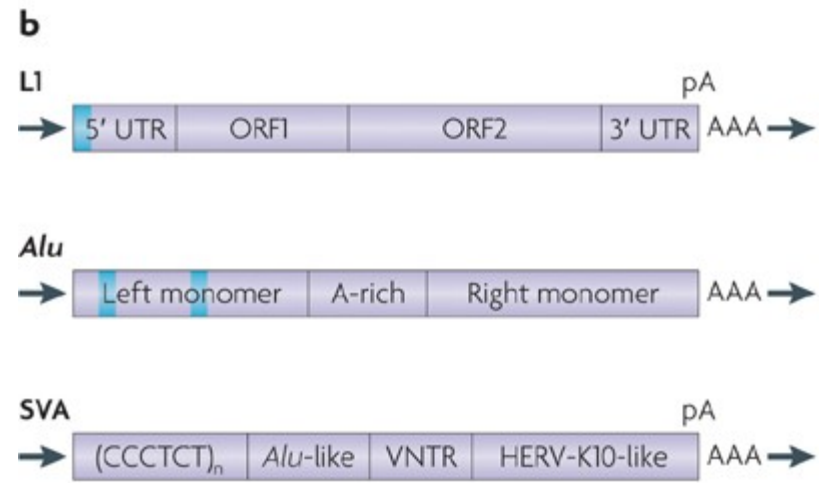
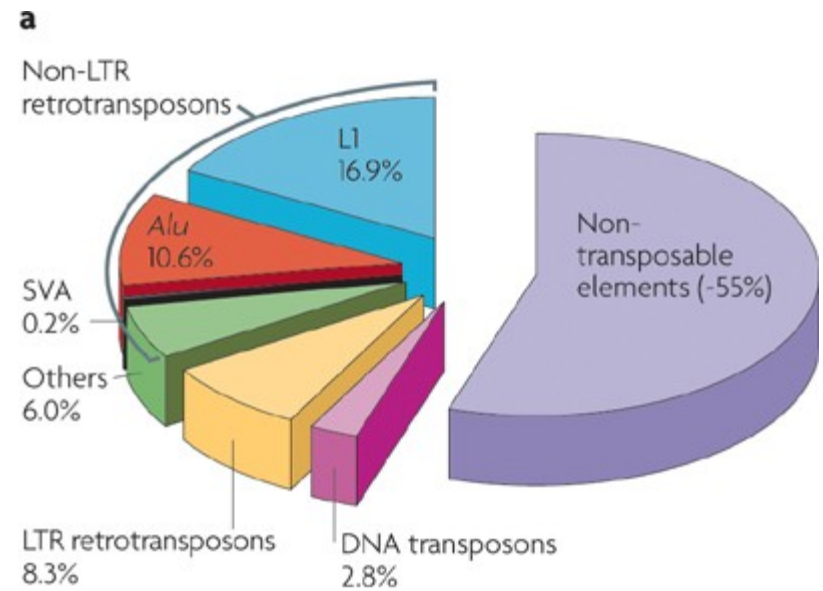
Newly described rearrangements are in bold letters; NAHR: nonallelic homologous recombination; NHEJ: nonhomologous end joining.

**V současné době je známo 117 typů velkých genomových
přeuspořádání v *LDLR* genu :
100 delecí a 17 duplikací.**

- *LDLR* gen obsahuje 98 *Alu* repetice, *Alu* repetice vytváří 65% intronových sekvencí.
- Vznik velkých genomových přeuspořádání v *LDLR* genu je asociován s *Alu* repeticemi.

- 45% lidského genomu je tvořeno transposabilními elementy.
- Alu repetice vytváří 10% lidského genomu; počet kopií je 1.3 million. Délka *Alu* repetice je 300 bp; složena ze dvou příbuzných monomerů; do genomu inzerována prostřednictvím ssRNA, která je transkribována RNA polymerázou III. 3'-konec *Alu* dimeru je ukončen oblastí bohatou na A (100 pb), monomery jsou odděleny oblastí bohatou na A,

• **Distribuce *Alu* repetice v genomu vytváří podmínky pro non-allelic homologous recombination (NAHR).**

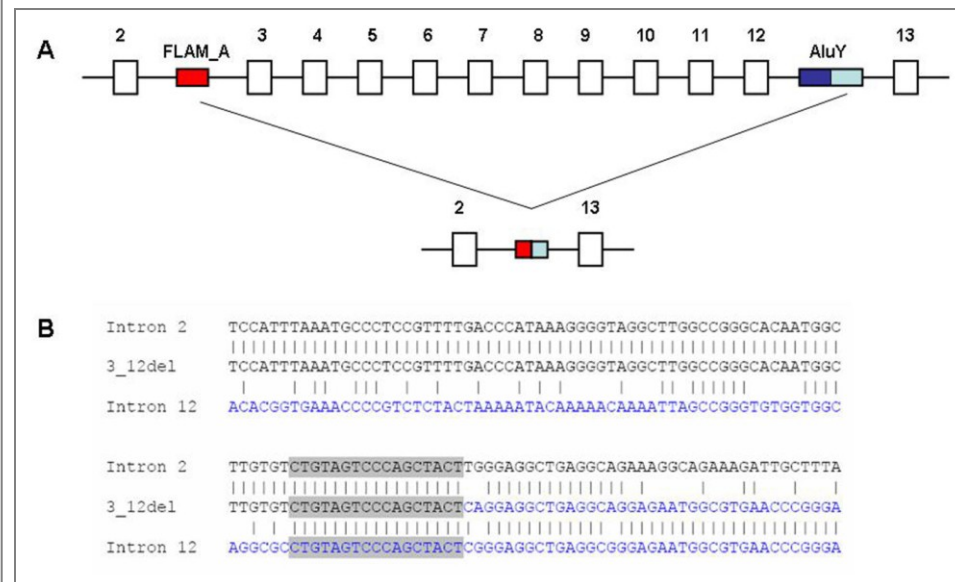
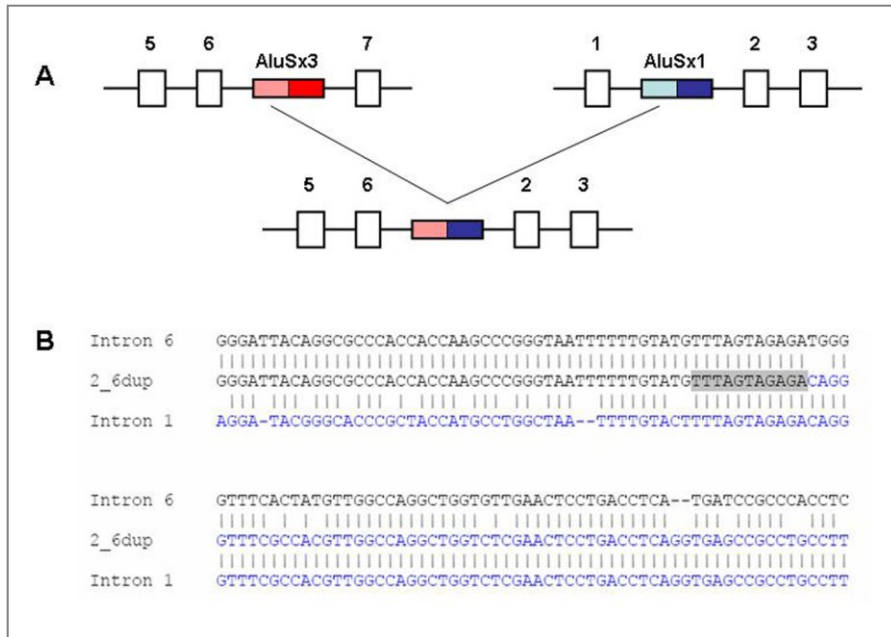


Výsledky analýzy 8 genových přeuspořádání u detekovaných u pacientů s FH

NAHR, non-allelic homologous recombination – detekováno u 4 delecí a 2 duplikací
 r.(promoter_ex2del), r.(ex2_6dup), r.(ex3_12del), r.(ex9_14del), r.(ex9_15del), r.(ex16_18dup)]

- 6 delecí/duplikací **rekombinace mezi dvěma *Alu* dimery**; detekce výrazné sekvenční identity v oblasti zlomu

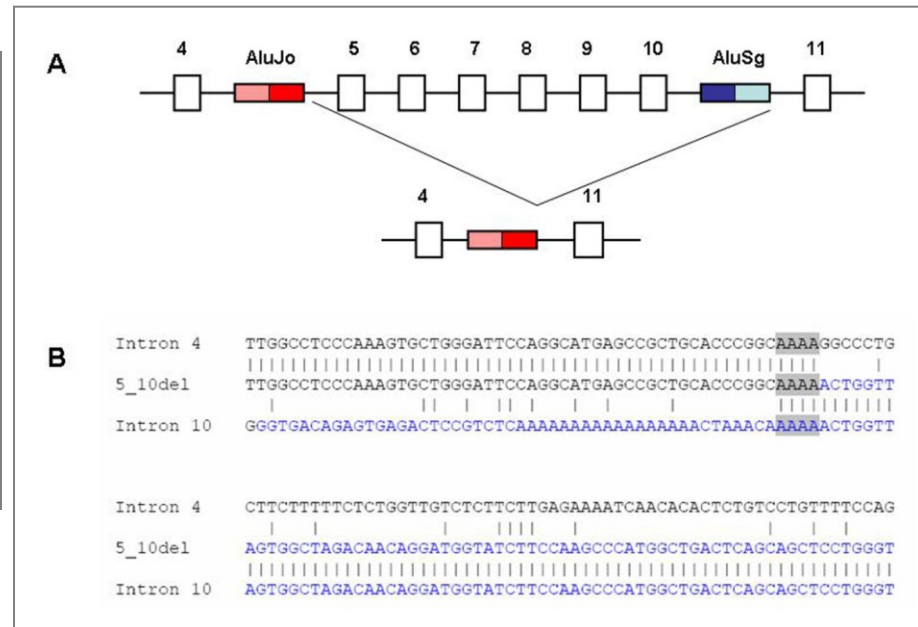
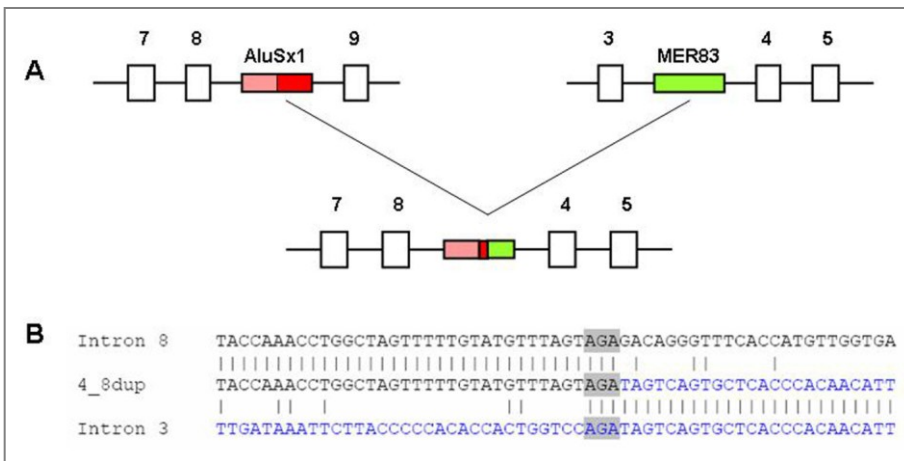
- 2 delece/duplikace rekombinace mezi ***Alu* monomermem a *Alu* dimerem**; detekce sekvenční identity v oblasti zlomu



NHEJ, nonhomologous end joining (zlomy v dsDNA, následuje spojení konců bez sekvenční homologie mezi rekombinovanými úseky DNA)

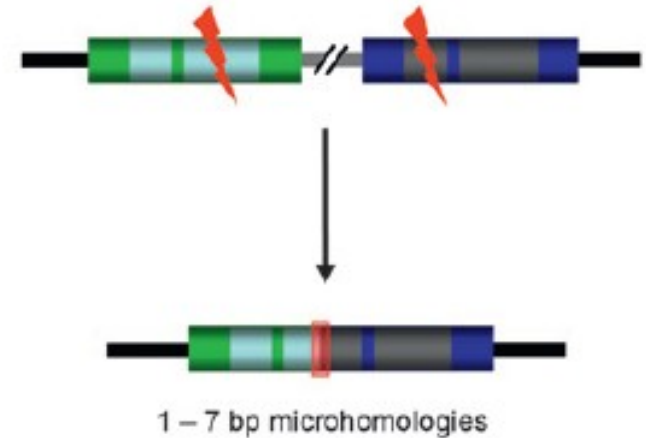
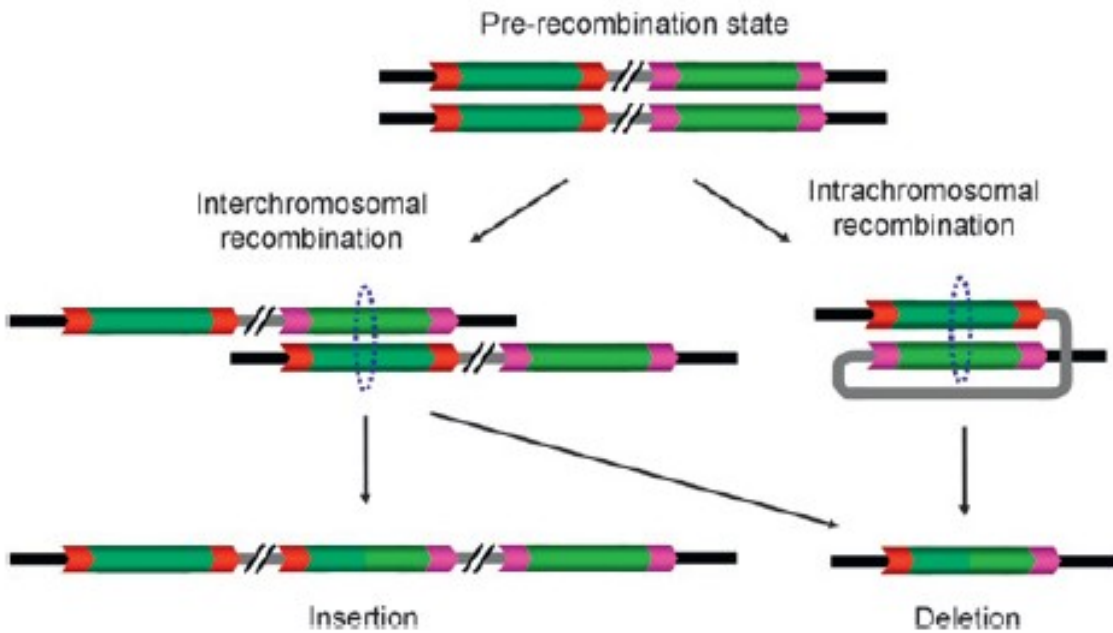
- Duplikace r.(ex4_8dup) – zlom lokalizovaný v intronu 3 je přítomen v *MER83* repetici; zlom lokalizovaný v intronu 8 je v *AluSx1*.

- Delece r.(ex5_10del) – zlom lokalizovaný v intronu 4 je přítomen na konci *AluJo* v antisense orientaci; zlom lokalizovaný v intronu 10 je na konci *AluSx1* v sense orientaci.



Nonallelic homologous recombination-mediated insertion/deletion (NAHR)

Nonhomologous end-joining-mediated deletion (NHEJ)



NAHR: rectangles depict directly orientated repeats sharing high homology (97% to 98%), which align at non-allelic and allelic positions and the subsequent recombination causes deletion and duplication. NHEJ: double-strand breaks (DSB) are created between the two sequences with no homology between each other. The NHEJ system modifies and rejoins the two ends, resulting in the deletion of the segment between the two DSBs. *Genome Res.* 2009 19: 1516-1526