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2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA

Jak interpretovat genetické vyšetření v éře sekvenování nové generace

Tereza Rašpličková

[Ü] BLOG
ústav biologie a lékařské genetiky

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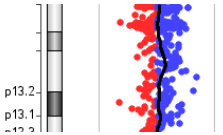

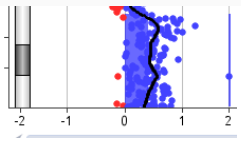

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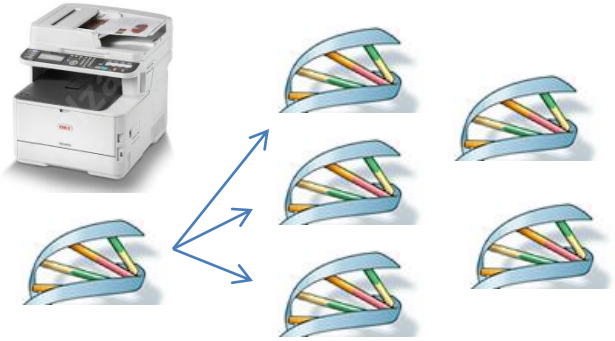
NGS - **N**ext **G**eneration **S**equencing

- *Sekvenování nové generace* – není jedna metoda, ale soubor nových technologií
- Masivní paralelní sekvenování - sekvenování až milionů sekvencí DNA současně
- Obrovský objem výstupních dat
- **Odhalení příčiny/predispozice k onemocnění**

  (studium genetické variability, analýza biologické diverzity…)

Fragmentace DNA



Amplifikace fragmentů DNA – vytvoření „klonů“

Masivní paralelní sekvenace DNA „klonů“ – určení pořadí nukleotidů v DNA

Sekvenování celého genomu/exomu najednou

Bioinformatická analýza dat

Interpretace nalezených variant

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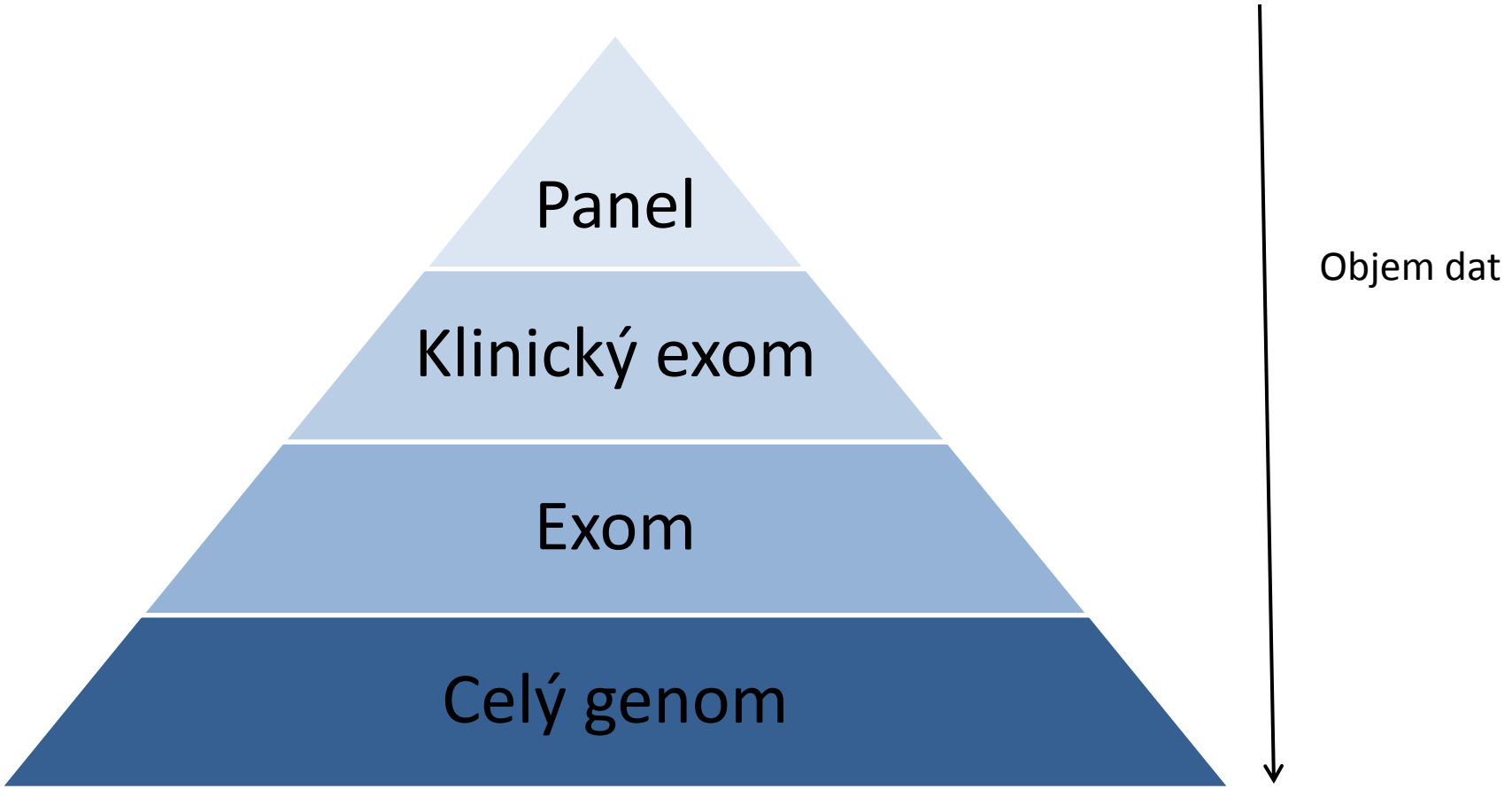
Co sekvenujeme?



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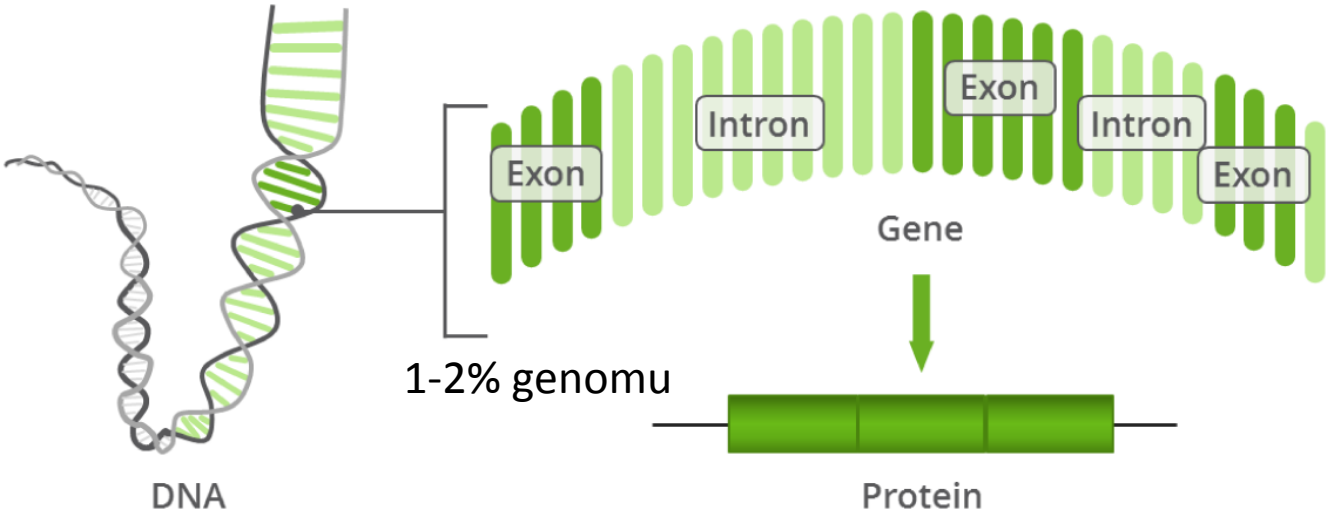
Panely – sekvenace vybraných genů

a další

Klinický exom, exom

- Exom = soubor exonů jednotlivých genů
- Sekvenace exomu = sekvenace protein-kódujících oblastí genomu
- Klinický exom obsahuje exony většiny genů spojených s onemocněními



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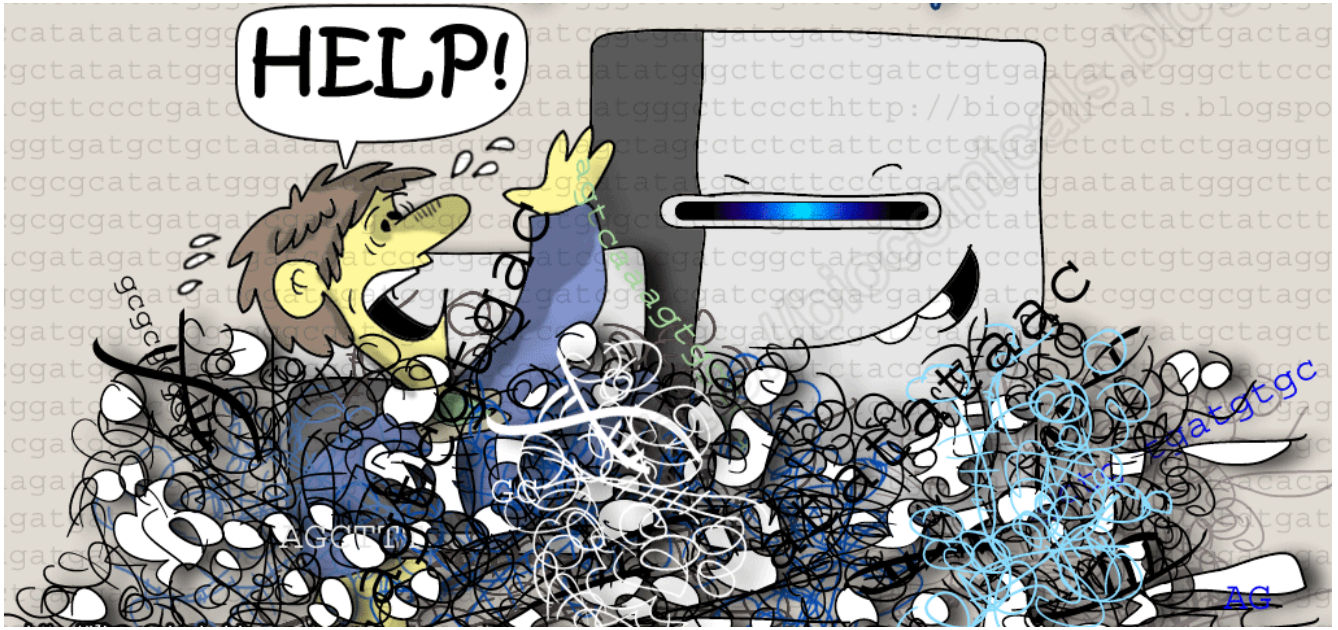
Celogenomové sekvenování

- Sekvenace kompletního genomu včetně nekódujících oblastí a mimojaderné DNA



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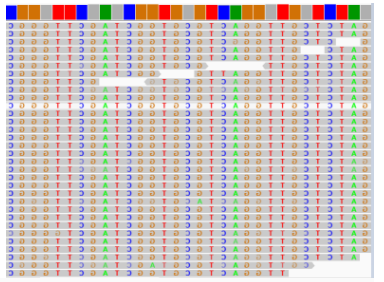
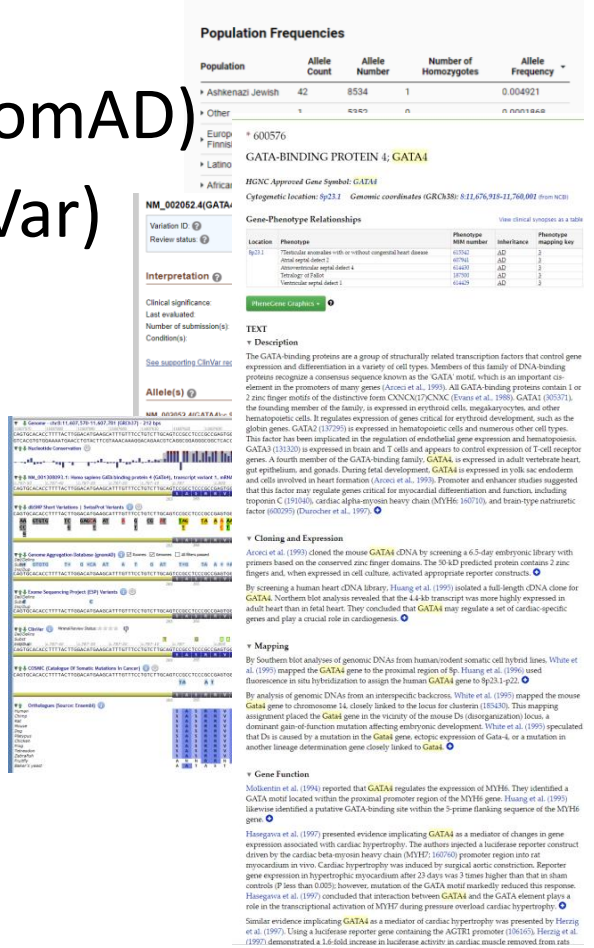
Interpretace variant

- Kvalita a hloubka čtení
- Frekvence výskytu v populaci (ExAC, gnomAD)
- Výskyt v mutační databázi (HGMD, ClinVar)
- Konzervovanost, vliv na protein
- Dědičnost
- Korelace genotyp-fenotyp
- Klasifikace variant (ACMG guidelines)
- Predikční softwary (PolyPhen-2, SIFT, MutationTaster…)





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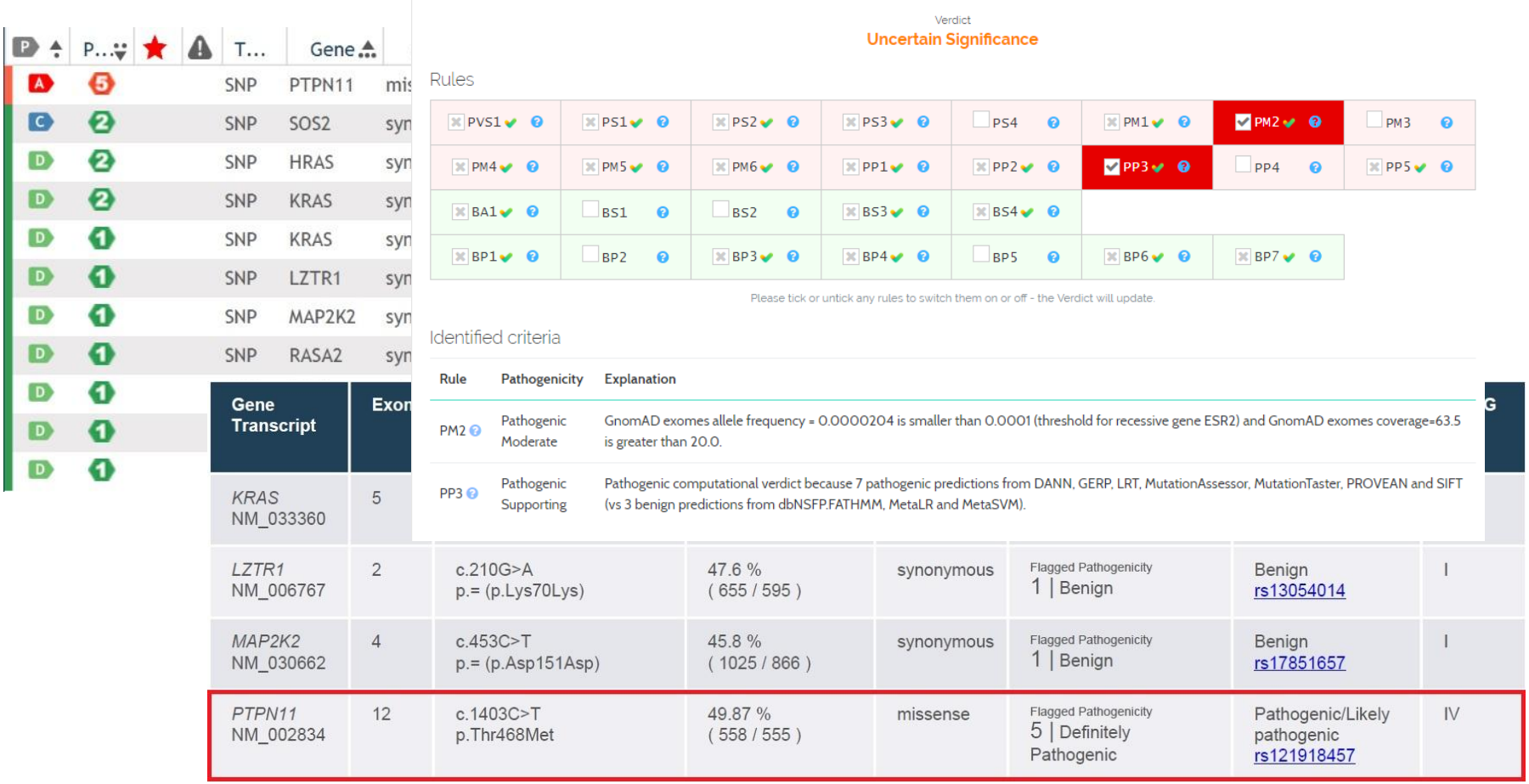
Filtrační softwary - komerční PC programy



P...		T...	Gene	
A	5	SNP	PTPN11	mis
C	2	SNP	SOS2	syn
D	2	SNP	HRAS	syn
D	2	SNP	KRAS	syn
D	1	SNP	KRAS	syn
D	1	SNP	LZTR1	syn
D	1	SNP	MAP2K2	syn
D	1	SNP	RASA2	syn
D	1			
D	1			
D	1			

Verdict
Uncertain Significance

Rules

PVS1	PS1	PS2	PS3	PS4	PM1	PM2	PM3
PM4	PM5	PM6	PP1	PP2	PP3	PP4	PP5
BA1	BS1	BS2	BS3	BS4			
BP1	BP2	BP3	BP4	BP5	BP6	BP7	

Please tick or untick any rules to switch them on or off - the Verdict will update.

Identified criteria

Rule	Pathogenicity	Explanation
PM2	Pathogenic Moderate	GnomAD exomes allele frequency = 0.0000204 is smaller than 0.0001 (threshold for recessive gene ESR2) and GnomAD exomes coverage=63.5 is greater than 20.0.
PP3	Pathogenic Supporting	Pathogenic computational verdict because 7 pathogenic predictions from DANN, GERP, LRT, MutationAssessor, MutationTaster, PROVEAN and SIFT (vs 3 benign predictions from dbNSFP.FATHMM, MetaLR and MetaSVM).

Gene Transcript	Exon						
KRAS NM_033360	5						
LZTR1 NM_006767	2	c.210G>A p.= (p.Lys70Lys)	47.6 % (655 / 595)	synonymous	Flagged Pathogenicity 1 | Benign	Benign rs13054014	I
MAP2K2 NM_030662	4	c.453C>T p.= (p.Asp151Asp)	45.8 % (1025 / 866)	synonymous	Flagged Pathogenicity 1 | Benign	Benign rs17851657	I
PTPN11 NM_002834	12	c.1403C>T p.Thr468Met	49.87 % (558 / 555)	missense	Flagged Pathogenicity 5 | Definitely Pathogenic	Pathogenic/Likely pathogenic rs121918457	IV

ACMG guidelines

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence	Multiple lines of computational	Novel missense change at an amino acid residue	Same amino acid change as an	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies sho no deleterious effect BS3					
Segregation data	Nonsegregation with disease BS4		members PP1			
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

- Benigní (Class 1)
- Pravděpodobně benigní (Class 2)
- Nejasná klinická signifikance (Class 3)
- Pravděpodobně patogenní (Class 4)
- Patogenní (Class 5)

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Výsledková zpráva – nalezené varianty

- Gen, transkript *GATA4*, NM _001308093.1 (NG_008177.2; NP_001295022.1)

- Typ mutace – zápis cDNA NM_001308093.1:c.196G>A, gDNA Chr8(GRCh37):g.11566017G>A, protein p.(Ala66Thr)
  - missense c.196G>A p.(Ala66Thr) p.A66T
  - samesense c.1149G>A p.(Thr383Thr) p.T383=
  - nonsense c.439G>T p.(Glu147Term) p.E147*
  - delece c.139_141delTCC p.(Ser47del) p.S47del
  - duplikace c.366_368dupCGC p.(Ala126dup) p.A126dup
  - inzerce c.341_342insA p.(Gly115Argfs*96) p.G115Rfs*96
  - intron c.998-269G>T p.(?)

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Výsledková zpráva – nalezené varianty

- Umístění exon 1, chr8:11566017
- Zygozita (heterozygot/homozygot/hemizygot)
- Klasifikace ACMG
- Onemocnění, dědičnost
- Původ (maternální/paternální/*de novo*)
- Seznam analyzovaných genů, použité testování

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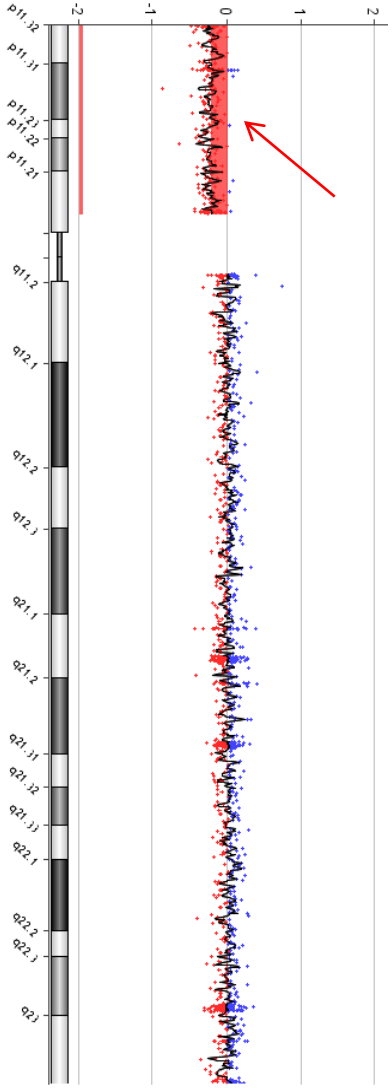
-2
-1
0
1
2
p11.32
p11.31
p11.23
p11.22
p11.21
q11.2
q12.1
q12.2
q12.3
q21.1
q21.2
q21.31
q21.32
q21.33
q22.1
q22.2
q22.3
q23


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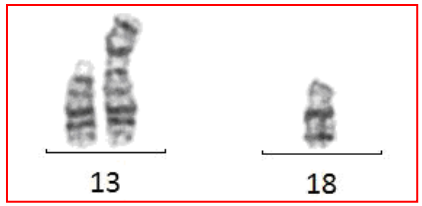
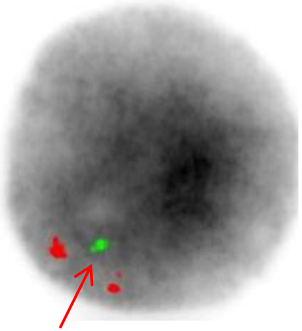
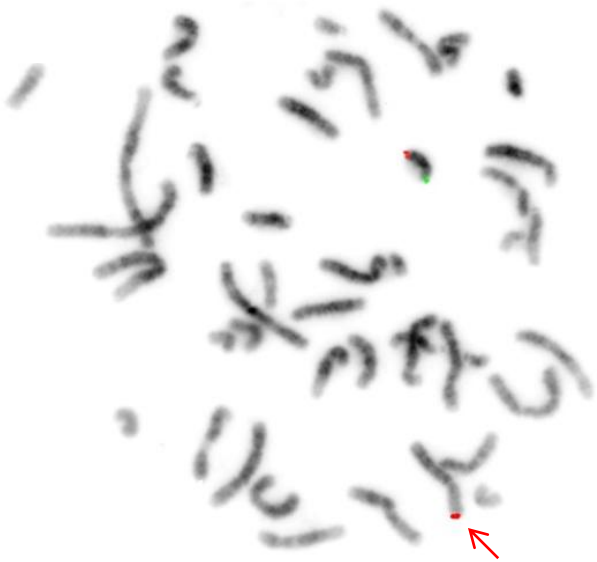
22
X
Y


13
18





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Více dat→ více variant

sdílení dat

spolupráce

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Děkuji za pozornost!

Poděkování

MUDr. Alice Krebsová, Ph.D.

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prim. MUDr. Markéta Havlovicová

Mgr. Románková Věra

Mgr. Jana Zarzycká

Mgr. Pavel Votýpka

MUDr. Anna Křepelová, CSc.

Zdroje

- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E et al. (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–423. doi: 10.1038/gim.2015.30
- https://www.genome.gov/27553432/editing-the-book-of-life-with-molecular-scissors/
- https://en.wikipedia.org/wiki/Nucleic_acid_sequence#/media/File:DNA_sequence.svg
- https://engenome.com/wp-content/uploads/2017/11/2017CardioVAI.pdf
- https://www.illumina.com/science/technology/next-generation-sequencing.html
- https://www.my46.org/intro/how-are-dna-changes-found
- https://www.alza.cz/oki-mc363dn-d4592545.htm?kampan=adpla_obecna_Tisk-a-Kancelar_tiskarny_laserove_c_1o5_9062881_PI020c3_~55283466701~&gclid=Cj0KCQiAtvPjBRDPARIsAJfZz0pEQHshste_7tPfER-UTmctRTtClaDP62KfEWuIirKkbEt4GKAv1E8aAonaEALw_wcB
- https://sequencing.com/what-is-whole-genome-sequencing-wgs
- https://news.expats.cz/czech-culture/prague-panelaks/
- https://dnalabsindia.com/blog/what-is-clinical-exome-sequencing/
- https://www.creative-biolabs.com/drug-discovery/therapeutics/whole-genome-sequencing-wgs.htm
- http://dineries.com/ca/mountainview/fiestadelmartoo/735villast/zoom
- http://biocomicals.blogspot.com/2011/07/
- http://3.bp.blogspot.com/-nDCf4vkSehE/TdNT0gALe4I/AAAAAAAADEI/RW-IVPBDfn4/s1600/collateral-damage.gif
- http://users.fred.net/tds/leftdna/icons/sherlock.jpeg
- http://bitesizebio.s3.amazonaws.com/wp-content/uploads/2016/02/08235812/DNA_sequence.jpg
- https://varsome.com/variant/hg19/syne2%3Ac.14831A%3EC
- https://skyoungleaders.com/finding-the-needle-in-the-leadership-haystack/