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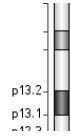


2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA

Jak interpretovat genetické vyšetření v éře sekvenování nové generace

Tereza Rašpličková





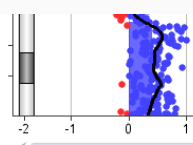
Genome browser interface showing coordinates from 234,000,000 to 234,000,000 and various tool icons.



FASTQ sequencing data for read 1, showing sequence alignment and quality scores.

FASTQ sequencing data for read 2, showing sequence alignment and quality scores.

FASTQ sequencing data for read 3, showing sequence alignment and quality scores.

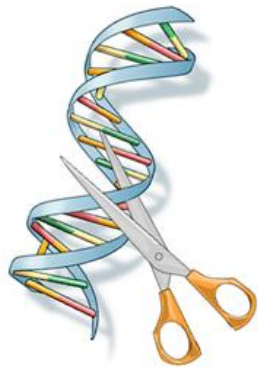




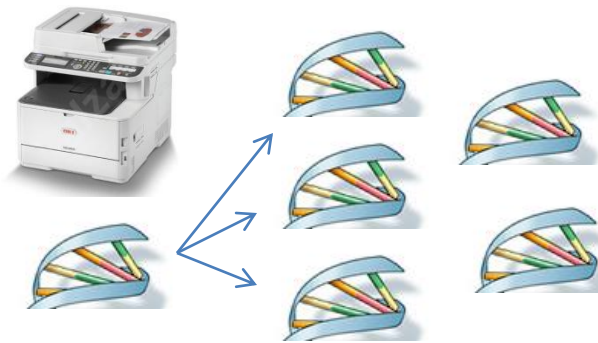
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NGS - Next Generation Sequencing

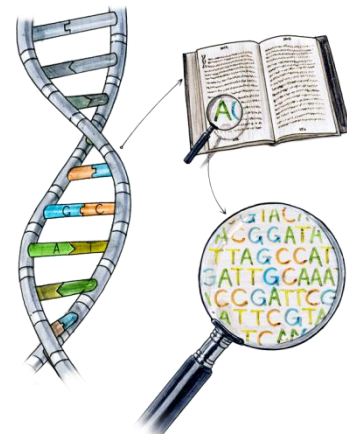
- *Sekvenování nové generace* – není jedna metoda, ale soubor nových technologií
- Masivní paralelní sekvenování - sekvenování až milionů sekvencí DNA současně
- Obrovský objem výstupních dat
- **Odhalení příčiny/predispozice k onemocnění**
(studium genetické variability, analýza biologické diverzity...)



Fragmentace DNA



Amplifikace fragmentů DNA – vytvoření „klonů“



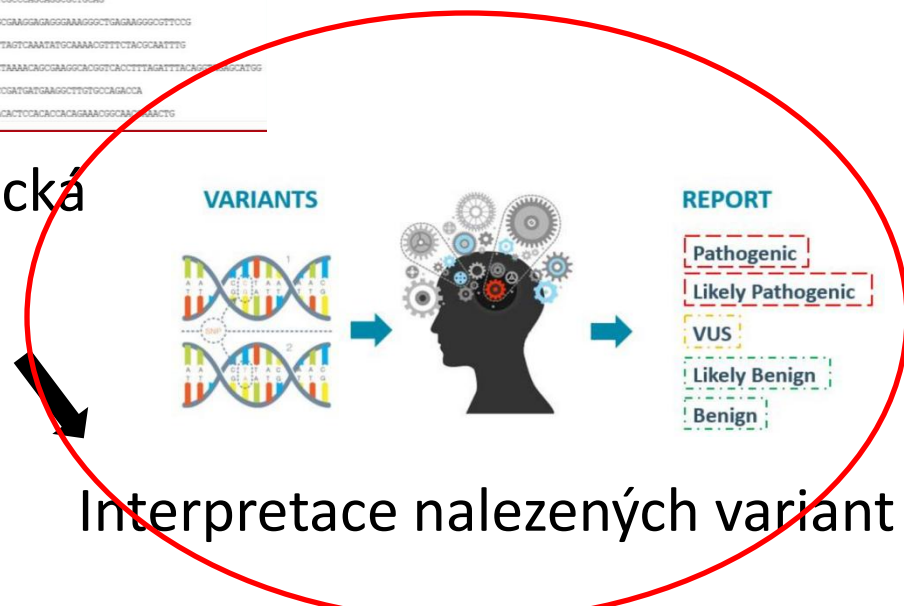
Masivní paralelní sekvenace DNA „klonů“ – určení pořadí nukleotidů v DNA



Sekvenování celého genomu/exomu najednou

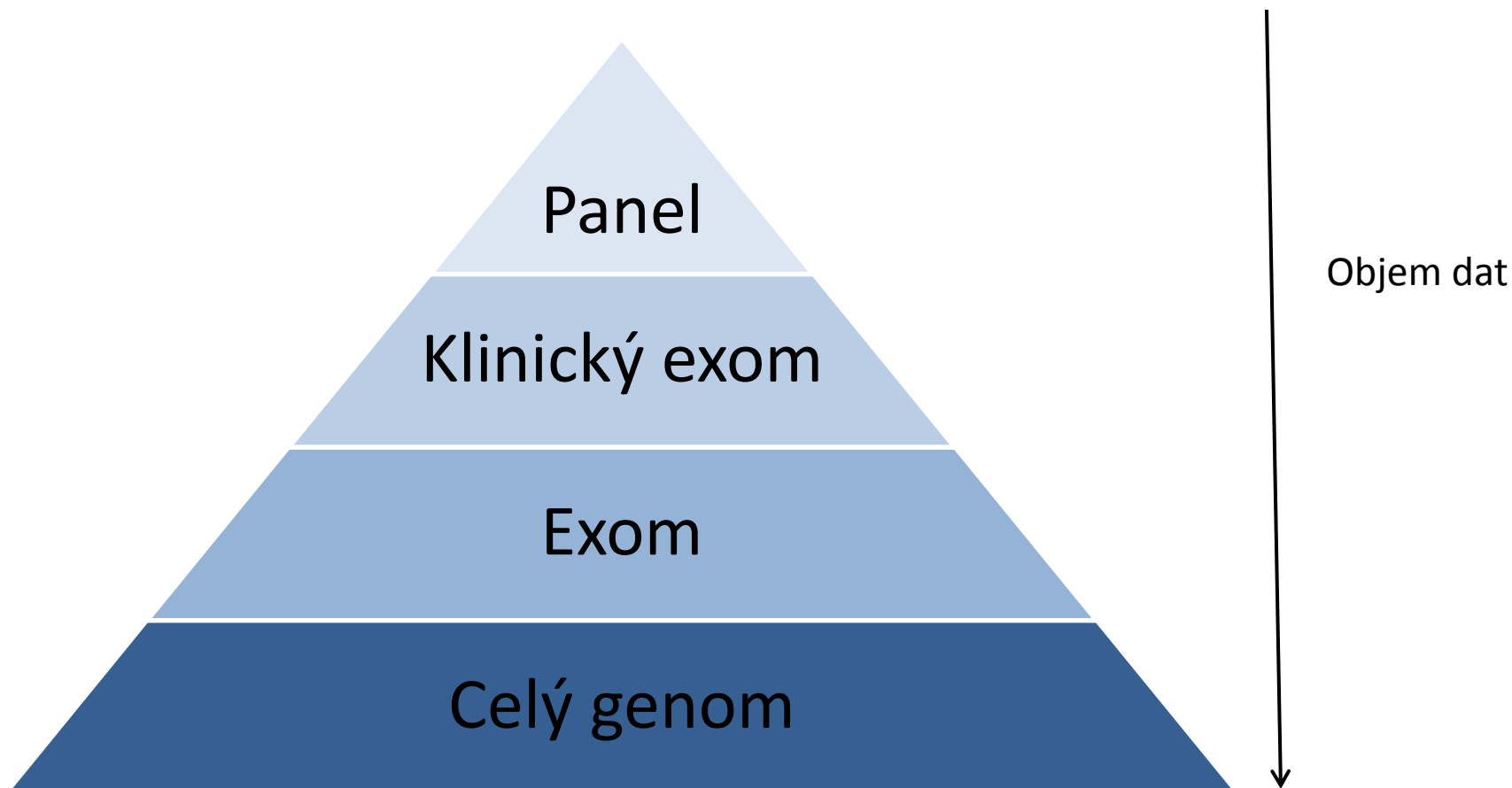


Bioinformatická analýza dat



Interpretace nalezených variant

Co sekvenujeme?





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Panely – sekvenace vybraných genů

aortopatie

kardiomyopatie

kanálopatie

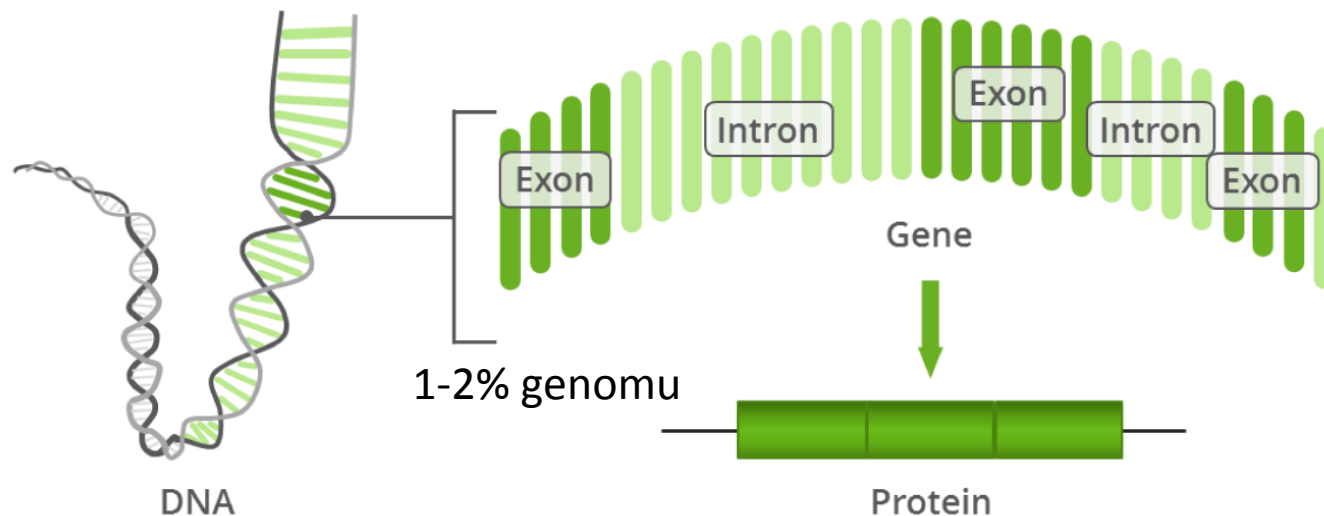
vrožené srdeční
vady



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

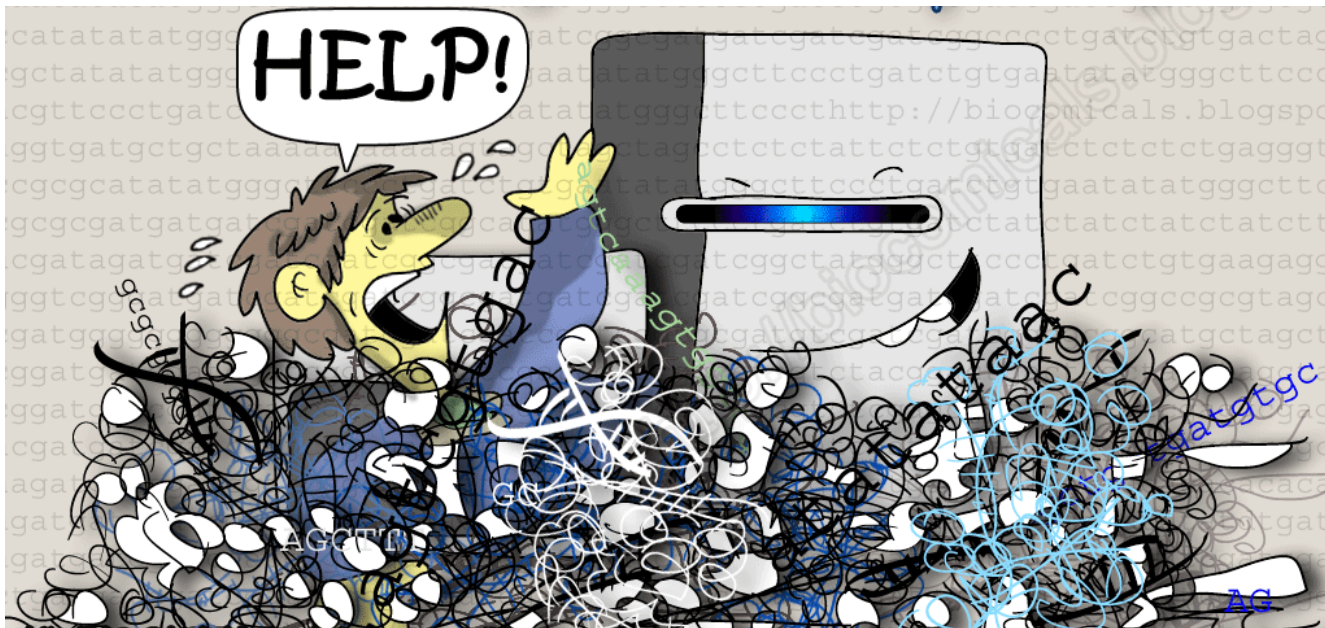




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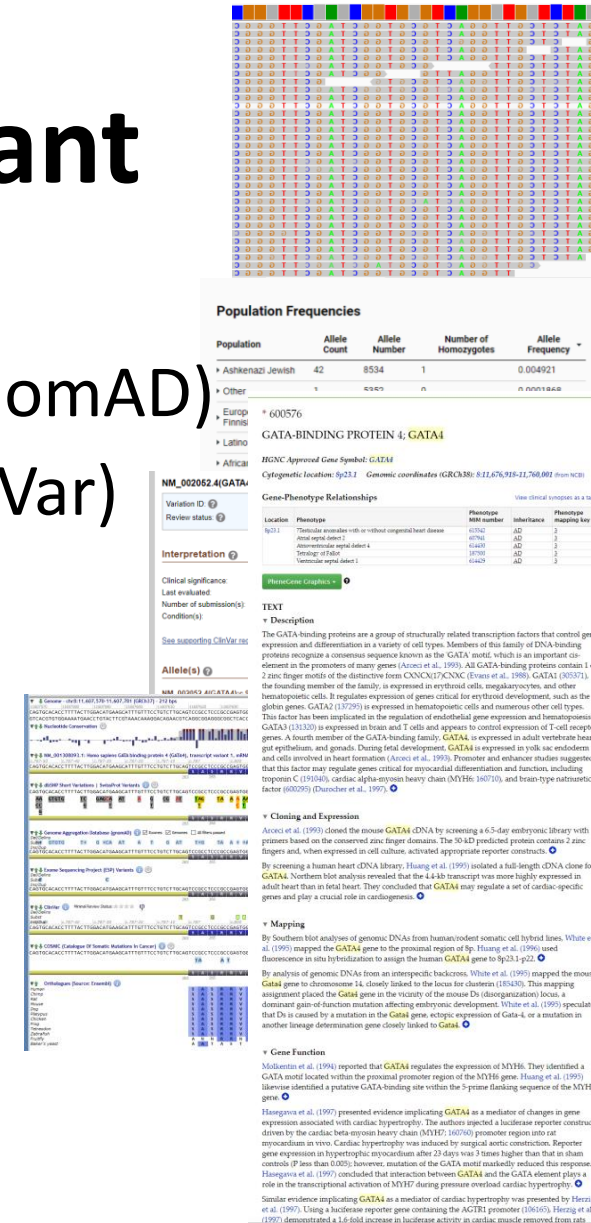
Celogenomové sekvenování

- Sekvenace kompletního genomu včetně nekódujících oblastí a mimojaderné DNA



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 softwaru (PolyPhen-2, SIFT, MutationTaster...)



Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Ashkenazi Jewish	42	1	1	0.004921
Other	1	1	0	0.001658

Gene-Phenotype Relationships

Location	Phenotype	Phenotype Ref ID	Phenotype Inheritance	Phenotype Frequency
9q21	"Distal aortic valve or without congenital heart disease	65192	AD	3
	Aortic septal defect	67761	AD	3
	Developmental septal defect 4	64440	AD	3
	Septum of valve	18700	AD	3
	Ventricular septal defect 1	14847	AD	3

Clinical significance

Interpretation: **Pathogenic**

TEXT: Description

The GATA binding proteins are a group of structurally related transcription factors that control gene expression and differentiation in a variety of cell types. Members of the family of DNA-binding proteins recognize a consensus sequence known as the GATA motif, which is an important cis-element in the promoters of many genes (Acucci et al., 1993). All GATA-binding proteins contain 1 or 2 zinc finger motifs of the distinctive form CXXCXXCXXC (Dress et al., 1988). GATA1 (16352) is the founding member of the family, is expressed in erythroid cells, megakaryocytes, and other hematopoietic cells. It regulates expression of genes critical for erythroid development, such as the globin genes. GATA2 (117293) is expressed in hematopoietic cells and numerous other cell types. This factor has been implicated in the regulation of endothelial gene expression and hematopoiesis. GATA3 (133320) is expressed in brain and T cells and appears to control expression of T-cell receptor genes. A fourth member of the GATA-binding family, GATA4, is expressed in adult vertebrate heart, gut epithelium, and gonads. During fetal development, GATA4 is expressed in yolk sac endoderm and cells involved in heart formation (Aizawa et al., 1993). Promoter and enhancer studies suggested that this factor may regulate genes critical for myocardial differentiation and function, including troponin C (191040), cardiac alpha-myosin heavy chain (MYH6 160710), and brain-type natriuretic factor (80020) (Duncker et al., 1995).

Cloning and Expression

Acucci et al. (1993) cloned the mouse GATA4 cDNA by screening a 6.3-day embryonic library with primers based on the conserved zinc finger domains. The 3042 predicted protein contains a zinc finger and, when expressed in oocytes, activated appropriate reporter constructs.

By screening a human heart cDNA library, Huang et al. (1995) isolated a full-length cDNA clone for GATA4. Northern blot analysis revealed that the 4.4-kb transcript was more highly expressed in adult heart than in fetal heart. They concluded that GATA4 may regulate a set of cardiac-specific genes and play a crucial role in cardiogenesis.

Mapping

By Southern blot analyses of genomic DNAs from human rodent somatic cell hybrid lines, White et al. (1995) mapped the GATA4 gene to the proximal region of 9p. Huang et al. (1996) used fluorescence in situ hybridization to assign the human GATA4 gene to 9q23.1-p22.

By analysis of genomic DNAs from an interspecific backcross, White et al. (1995) mapped the mouse Gata4 gene to chromosome 3, closely linked to the locus for diabetes (INS2). This mapping assignment placed the Gata4 gene in the vicinity of the mouse D3 (diabetogenesis) locus. A dominant gain-of-function mutation affecting embryonic development, White et al. (1995) speculated that D3 is caused by a mutation in the Gata4 gene, ectopic expression of Gata-4, or a mutation in another lineage determination gene closely linked to Gata4.

Gene Function

Molteni et al. (1994) reported that GATA4 regulates the expression of MYH6. They identified a GATA motif located within the proximal region of the MYH6 gene. Huang et al. (1995) likewise identified a putative GATA-binding site within the 5-prime flanking sequence of the MYH6 gene.

Huang et al. (1997) presented evidence implicating GATA4 as a mediator of changes in gene expression associated with cardiac hypertrophy. The authors injected a luciferase reporter construct driven by the cardiac beta-myosin heavy chain (MYH7 160700) promoter region into rat myocardium in vivo. Cardiac hypertrophy was induced by surgical aortic constriction. Reporter gene expression in hypertrophic myocardium after 23 days was 3 times higher than that in sham controls (P less than 0.005); however, mutation of the GATA motif markedly reduced this response. Huang et al. (1997) concluded that interactions between GATA4 and the GATA element play a role in the transcriptional activation of MYH7 during pressure overload cardiac hypertrophy.

Similar evidence implicating GATA4 as a mediator of cardiac hypertrophy was presented by Hering et al. (1997). Using a luciferase reporter gene containing the ACTB1 promoter (160365), Hering et al. (1997) demonstrated a 16-fold increase in luciferase activity in cardiac muscle removed from rats

Filtrační softwary - komerční PC programy

P	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

<input checked="" type="checkbox"/> PVS1	<input checked="" type="checkbox"/> PS1	<input checked="" type="checkbox"/> PS2	<input checked="" type="checkbox"/> PS3	<input type="checkbox"/> PS4	<input checked="" type="checkbox"/> PM1	<input checked="" type="checkbox"/> PM2	<input type="checkbox"/> PM3
<input checked="" type="checkbox"/> PM4	<input checked="" type="checkbox"/> PM5	<input checked="" type="checkbox"/> PM6	<input checked="" type="checkbox"/> PP1	<input checked="" type="checkbox"/> PP2	<input checked="" type="checkbox"/> PP3	<input type="checkbox"/> PP4	<input checked="" type="checkbox"/> PP5
<input checked="" type="checkbox"/> BA1	<input type="checkbox"/> BS1	<input type="checkbox"/> BS2	<input checked="" type="checkbox"/> BS3	<input checked="" type="checkbox"/> BS4			
<input checked="" type="checkbox"/> BP1	<input type="checkbox"/> BP2	<input checked="" type="checkbox"/> BP3	<input checked="" type="checkbox"/> BP4	<input type="checkbox"/> BP5	<input checked="" type="checkbox"/> BP6	<input checked="" type="checkbox"/> 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	Variant	Frequency	Effect	Pathogenicity	Reference	Grade
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 show 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 <i>trans</i> with a dominant variant BP2 Observed in <i>cis</i> with a pathogenic variant BP2		For recessive disorders, detected in <i>trans</i> 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.(?)



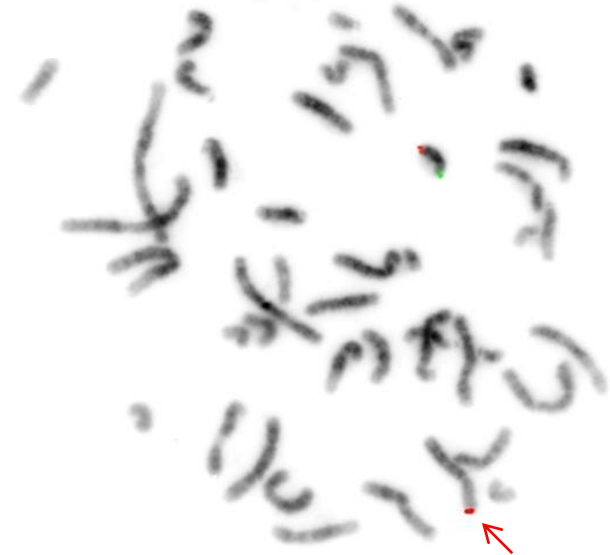
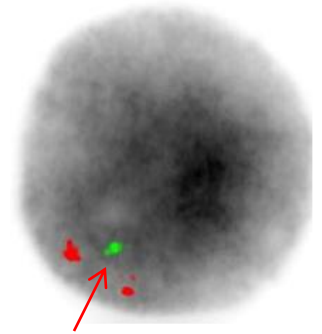
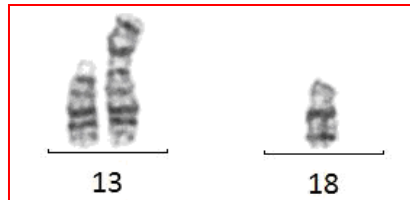
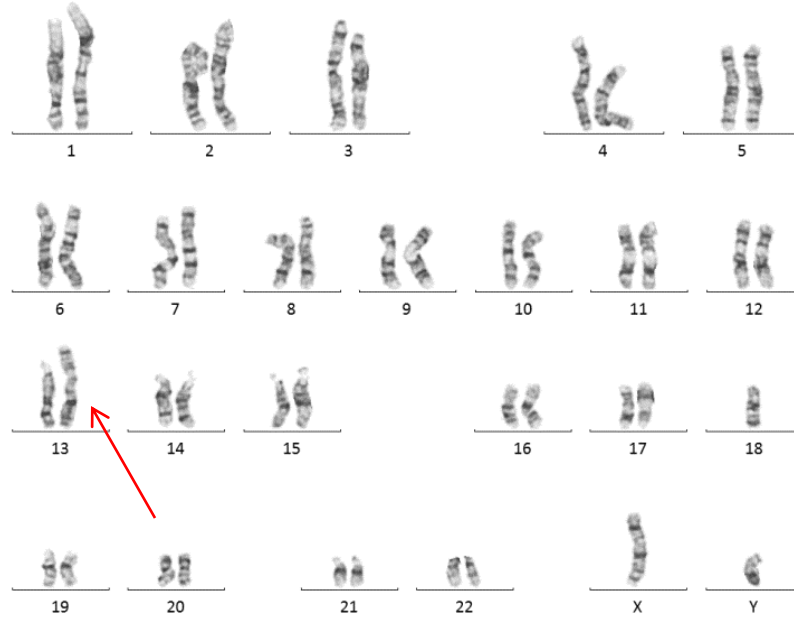
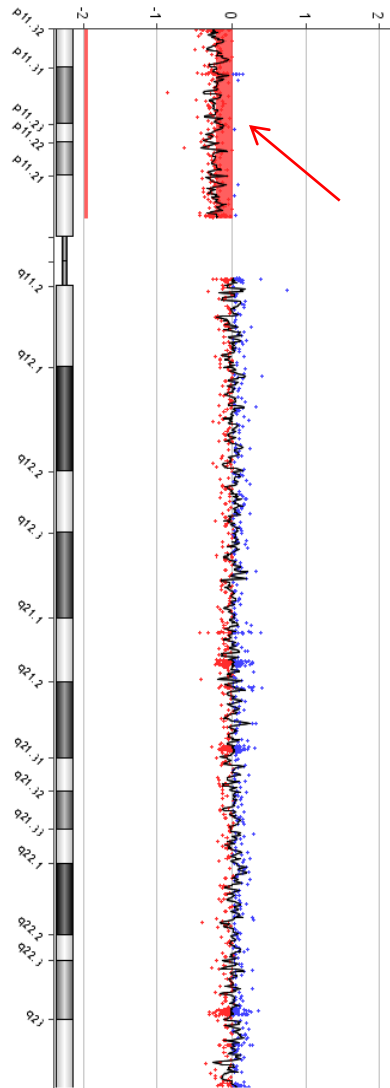
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Výsledková zpráva – nalezené varianty

- 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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Více dat → více variant



sdílení dat

spolupráce



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Děkuji za pozornost!

Poděkování

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Zdroje

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