Molecular genetic diagnostics of monogenic diseases

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Molecular genetic diagnostics of rare diseases:

- Neuromuscular diseases
- Epilepsies
- Skin diseases
- Connective tissue diseases
- Metabolic diseases

Molecular genetic diagnostics of monogenic diseases

Why are we actually finding out?

1. Confirmation of clinical diagnosis

- > psychological support
- > prediction of the course of the disease
- > specific treatment certain disease, certain mutation (example at the end of the lecture)

2. Segregation of variants/disease in family members

- early treatment (in preclinical phase)
- > genetic counseling testing of partner, preimplantation diagnostics, prenatal diagnostics

Molecular genetic diagnostics of monogenic diseases

Germline mutation/mutations in **one gene**, not large deletions/insertions containing several genes:

- identification of small scale variants: nucleotide substitutions, small deletions / insertions
- whole exon deletions / duplications (copy number variations, CNV) Aberrant spli



Molecular genetic diagnostics of monogenic diseases

Germline mutation/mutations in one gene, not large deletions/insertions containing several genes:

- identification of small scale variants: nucleotide substitutions, small deletions / insertions
- whole exon deletions / duplications (copy number variations, CNV)



Deletion of one exon:

Molecular genetic diagnostics of monogenic diseases

Material: DNA isolated from the whole blood

- 1. Classic Sanger sequencing
- 2. Next generation sequencing
- 3. MLPA CNV detection
- 4. RP-PCR detection of repeat expansions
- 5. Southern blot and hybridization detection of repeat expansions / deletions

1. Classic Sanger sequencing

identification of small scale variants: nucleotide substitutions, \succ small deletions / insertions

> Method description:

PCR (polymerase chain reaction, amplification of known target sequence) > sequencing

3. result

73





A. Sequencing of the certain part of the gene including the position of pathogenic variant

- segregation of variant in family members
- B. Sequencing of the whole gene by several PCR reactions:
 - in past: gene by gene approach (time-consuming and costly)
 - gene with clear clinical-genetic relationship, not a very long gene (example: phenylketonuria)

1. Classic Sanger sequencing

A. Sequencing of the certain part of the gene including the position of pathogenic variant

segregation of variant in family members

Patient with **autosomal recessive** limb girdle muscular dystrophy, 2 pathogenic variants in *CAPN3* gene *CAPN3*: c.245C>T and c.550delA

Presence of variants in:

- mother
- father
- brother
- sister





B. Sequencing of the whole gene: gene with clear clinical-genetic relationship, not a very long gene example: phenylketonuria

Phenylketonuria (PKU)

autosomal recessive metabolic disease (deficiency of phenylalanine hydroxylase); gene PAH (12q23.2)

- diagnosed by newborn screening, on the basis of increased phenylalanine and the ratio phenylalanine/tyrosine
- increased Phe and Phe/Tyr is the indicator for DNA analysis of the PAH gene encoding the phenylalanine hydroxylase
- in 98% of cases, two pathogenic variants in the *PAH* gene are identified
 = clinical diagnosis of PKU is confirmed





Molecular genetic diagnostics of monogenic diseases

- 1. Classic Sanger sequencing
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Material: DNA isolated from the whole blood

2. Next generation sequencing (NGS)

- identification of small scale variants: nucleotide substitutions, small deletions
 / insertions
- whole exon deletions / duplications (copy number variations, CNV)
- > Method description:
 - targeted panel sequencing detection of variants in selected sets of genes or gene regions (coding regions)
 - whole exome sequencing (WES)
 - whole genome sequencing (WGS)

> Principle - DNA samples are converted into sequencing libraries

- DNA is randomly sheared into smaller fragments by mechanical or enzymatic methods
- adapters for sequencing and multiplexing are added to DNA ends
- regions of interest within the library are captured using oligonucleotide probes (hybridization)
- probe-targeted fragment complex is separated from other fragments that are not bound to probes
- amplification of targeted regions
- NGS of targeted regions



2. Next generation sequencing (NGS)

Muscular dystrophies and myopathies

- to date, 162 genes associated with clinical manifestation of muscular dystrophy/myopathy
- clinical, biochemical, pathological,.... findings are mostly not specific enough for selection of a gene for molecular genetic analysis
- Which gene to analyse?

In past before NGS:

- genes analysed sequentially by classical DNA sequencing
- starting with a gene with the most likely mutation occurrence > negative result > another gene
- TIME AND FINANCIALLY CONSUMING
- only a certain number of genes analysed

NGS era:

- all genes associated with the disease analysed at the same time (in parallel) = targeted panel
- FAST AND RELATIVELY CHEAP

Result:

Identification of a large number of sequence variants

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dAJ91581	1287	171	~ 7	1E+08 SNV	6		No			189	217	0.4655	406	408 A/A	No	EDAR	ectodyspl EDAR	NM 022336.3 ± 1056CsT	NM 0223 No	~	~	not speci C		
dAJ91581	1314	172	2	15+08 SNV	G	A	No	Homozyg	100	193	189	0.4948	382	382 A/A	No	EDAR		NM 022336.3 c 750C T	NM 022: No			not spec C		
dAJ91581	1314	173	2	1E+08 SNV	A	G	No	Homozyg	100	272		0.4982	542	542 G/G	No	EDAR		NM 022336.3 c 655+30TxC						significanc
dAJ91581	120	174	2	15+08 SNV	с	т	No	Heterozys	48,092	107	90	0,4569	197	404 C/T	No	ERCC3	ERCC excl ERCC3	NM_000122.1:c.*259GxA; N	NM_0011-			0,0182 Xeroderm L	ikely_bei	15/15, 15/
dAJ9158(82	175	2	2E+08 SNV	с	т	No	Heterozys	49,072	138	133	0,4908	271	563 C/T	No	ACVR1	activin A r ACVR1	NM_001105.4:c.*138GxA; N	N NM_001: -			0,0821 Progressi 8	enign	12/12, 11/
dAJ9158(178	176	2	2E+08 SNV	с	т	No	Homozyg	99,52	301	325	0,4808	626	628 T/T	No	ACVR1	activin A r ACVR1	NM_001105.4:c.690GxA; NM	/ NM_001: No			Progressi B	enign	8/12,7/11
dAJ9158	166	177	2	2E+08 SNV	G	A	No	Heterozyg	48,958	145	141	0,493	286	582 A/G	No	ACVR1	activin A r ACVR1	NM_001105.4:c.270CsT; NN				Progressi B	enign 1	5/12, 4/11
dAJ9158	723	178	2	2E+08 Deletion		-	No	Heterozyg	47,222	120	106	0,469	227	480 complex		ITG86	integrin s ITG86	NM_000888.4:c.2269-5del;				not_spec 8		
dAJ9158	306	179	2	1609583 Deletion			No		47,239	114	114	0,5	229	486 complex		ITG86	integrin s ITG86	NM_000888.4:c.2269-6_22				not_spec 8		
dAJ9158f	9	180	2	28+08 SNV	с	T	No	Heterozyg	49,66	171	157	0,4787	328	659 C/T	No	ITG55	integrin s ITG86	NM_000! NP_0012 NP_000						8/13, 10/
dAJ9158f	636		2	2E+08 SNV	G	A	No		45,307				208	460 A/G	No	ITGA6	integrin s ITGA6	NM_000; NP_0013 NP_000		0,3126		0,2516 Epidermo B		7/26, 7/2
dAJ91584 dAJ91584	725	182	2	2E+08 SNV 2E+08 SNV	A .	G	No		51,724	70		0,4575	153 302	288 A/G 591 C/T	No	ITGA6	integrin s ITGA6	NM_000210.3:e.1487+7AsG		0,4081		0,4072 Epidermo B		10.00 10
AJ9158	400	185		25408 SNV 1733690 Insertion	С	-	No		51,263 37,104	150	152	0,4967	257	591 C/I 671 -/T	No	ITGA6	integrin s ITGA6 integrin s ITGA6	NM_000210.3:e.2082CsT; N NM_000210.3:e.*204_*205				0,3467 Epidermo B 0,1058 Epidermo U		
dAJ91581	801	185		2FLOB SNV		G	No		46,296	143		0,4509	811	670 A/G	No	ITGAS	integrin s ITGA6	NM_000210.3:c.*435AvG: N		0,1591	0,1090	0,1056 Epidermo E		
AU9158	705	185		1784047 Insertion	2	4446	No	Heterozy	59.77	26		0,4358	74	123 comoles		AGPS	alkyigiyce AGP5	NM 003659.3:c.*1814 *18				Rhizomel 5		20/20, 21
AJ9158	692	187		2E+08 SNV	4	G	No	Heterozys	44.828	10	10	0,5514	20	45 A/G	No	AGPS	alkyigiyee AGPS	NM 003659.3:c.*1866AvG				Rhizomeli B		20/20
AJ9158	67	188		1784048 Deletion	TA		No		39,535	16	11	0.4074	27	67 complex		AGPS	alkyigiyce AGPS	NM 003659.3:c.*1898 *18				And Other D		20/20
AJ9158	666	189	2	1784048 Deletion	TATA		No	Heterozys	46,512	17	13	0.4333	30	67 complex		AGPS	alkylglyce AGPS	NM 003659.3 c.*1896 *18				Rhizomeli C	onflictin	20/20
AJ9158	704	190	2	2E+08 SNV	c	т	No	Heterozya	46,784	114	130	0.4672	244	510 C/T	No	AGPS	alkylglyce AGPS	NM 003659.3 c.*3558CvT	NM 0036-			Rhizomel 8	lenign	20/20
AJ9158	740	191	2	1784067 Insertion	-	т	No	Heterozys	45,614	77	65	0,4577	142	320 -/T	Yes	AGPS	alkylglyce AGPS	NM_003659.3:c.*3854dup	NM_003(-			Rhizomel 8	lenign	20/20
AJ9158	75	192	2	1784068 Deletion	TTTTAG		No	Heterozys	43,621	70	73	0,4895	143	344 complex	No	AGPS	alkylglyce AGPS	NM_003659.3:c.*3917_*39	2 NM_003(-			Rhizomel U		20/20
AJ9158f	737	193	2	2E+08 SNV	С	A	No	Heterozyg	50,145	141	103	0,4221	244	500 A/C	No	AGPS	alkylglyce AGPS	NM_003659.3:c.*4253C+A	NM_003(-			Rhizomeli B	enign	20/20
AJ9158	738	194	2	2E+08 SNV	Ŧ	С	No	Heterozyg	43,258	120		0,4872	234	539 C/T	No	AGPS	alkylglyce AGPS	NM_003659.3:c.*4974TxC	NM_003(-			Rhizomeli B		20/20
AJ9158	341	195	2	2E+08 SNV	G	c	No		44,444	108		0,4953	214	493 C/G	No	COLSA1		NM_000090.3 c 1816-14G>		0,1667		0,129 Ehlers-Da B		
AJ9158	340	196	2	2E+08 SNV	G	A	No		44,828	102	97	0,4874	199	434 A/G	No	COL3A1		NM_000090.3:c1851GxA	NM_0000 No		0,1261	0,129 Ehlers-Da 8		26/51
AJ9158	628	197	2	26+08 SNV	т	с	No		47,826	95	95	0,5	190	400 C/T	No	COL5A1		NM_000090.3:c.2244TxC	NM_0000 No	0,285	0,5138			52/51
AJ9158	1523	198	2	28+08 SNV	т	G	No			290		0,4775	555	558 G/G	No	COL3A1		NM_000(NP_0000 NP_000				not_spec 8		50/51
AJ9158	519	199	2	1898772 Deletion	AG	•	No			149		0,4806	310	571 complex	No	COLSA1 COLSA2		NM_000090.3:c.*799_*800				0,2033 Ehlers-Da B		51/51
AJ9158	1321 1320	200	2	2E+08 SNV 2E+08 Deletion	A .	G	No	Homozyg		237		0,4936	205	470 G/G 245 -/-	No	COLSA2 COLSA2		NM_000393.4:c.3411TxC NM_000393.4:c.2086-12del	NM_0001 No					48/54
AJ9158	1520	201	2	25+08 Deletion	-	- C	No	Homozyg		290		0,4295	586	588 C/C	No	COL542		NM 000393.4 c 1311A-G	NM 0005 No			not_speci 8		21/54
AJ91581	1522	202	2	25+08 SNV	G	-	No	Homozyg	100	257			546	546 T/T	No	COLSAZ		NM 000395.4x:315CxA	NM 0001 No			not spec 8		2/54
AJ9158	997	203	2	25+08 SNV	T	c	No	Heterozys		103		0,4747	217	410 C/T	No			NM 015657.3:c.*790AxG: N				Consenita B		45/45 5
AJ9158	1311	205	;	2EHOB SNV	A	G	No		97,786	215		0.4459	388	394 6/6	No			NM 015657.3:c 3209+20Tx				not speci 8		
AJ91581	599	206	2	2E+08 SNV	T	c	No	Heterozya	49,588	192	191	0.4987	383	776 C/T	No			NM 015657.3 c 2079AvG: N		0.1855	0.2331	0 1955 Consenits B		14/45.2
AJ91584	1310	207	2	2E+08 SNV	4	Ť	No	Homozyg	99,237	224	157	0.4121	381	383 T/T	No	A8CA12	ATP bindi ABCA12	NM 015(NP 7750 NP 775	0 NM 173(Yes			Congenita 8	leging	9/45, 17
AJ91581	178	208	2	2E+08 SNV	c	T	No			199		0.4877	408	409 T/T	No	FN1	fibronect FN1	NM 0015 NP 9976 NP 997						39/44.3
AJ91581	1	209	2	25+08 SNV	С	т	No		50,327	183	188	0,4933	371	755 C/T	No	FN1	fibronect FN1	NM_001! NP_0012 NP_997	6 NM_2124 Yes					34/44, 3
AJ9158(175	210	2	2E+08 SNV	с	т	No	Heterozys	48,413	97	88	0,4757	185	378 C/T	No	FN1	fibronect FN1	NM_001306129.1:c.4725G						28/44, 2
AJ9158(126	211	2	26+08 SNV	G	с	No	Heterozys	51,351	52			115	229 C/G	No	FN1	fibronect FN1	NM_001306129.1:c.4253-1						
AJ9158	113	212	2	2E+08 SNV	С	т	No	Heterozyg	43,11	94	99	0,487	193	446 C/T	No	FN1	fibronect FN1	NM_001306129.1:c.3253+1						
AJ9158	113	213	2	26+08 SNV	т	G	No	Heterozyg	47,386	120			237	492 G/T	No	FN1	fibronect FN1	NM_001306129.1:c.3156A						20/44, 2
AJ9158	113	214	2	25+08 SNV	т	G	No			120		0,4894	235	445 G/T	No	FN1	fibronect FN1	NM_001306129.1:c.3111Ax						20/44, 2
AJ91581	178	215	2	28+08 SNV	т	G	No	Homozyg	100	284		0,4731	539	539 G/G	No	FN1	fibronect FN1	NM_001! NP_0012 NP_997						17/44, 1
AJ9158(AJ9158)	103	216		2162748 Insertion 2E+08 SNV	-	AC	No	Homozyg		136	164	0,4533	300	355 AC/AC	No	FN1 FN1	fibronect FN1	NM_001306129.1:c.1942-1						
		217	2	2E+08 SNV 2E+08 SNV	-	G	No		44,295	97		0,5	194	442 A/T 266 C/G	No	PN1	fibronect FN1	NM_0013 NP_0012 NP_997						1/44, 1/4
AJ91584 C AJ91584	424	218	2	25+08 SNV 25+08 SNV	c	T	No	Heterozyg	52,83 47,619	97	109	0,4015	206	266 C/G 451 C/T	No	Changes -	CHARME - CARPOOL	NM_001306129.1:c-270Gx NM_001127207.1:c-368Cs				0.2079 Schimke B		1/18
AJ91581	424	219	2	26+08 SNV	-	4	No	Heterozy	48,663	135	151	0,4709	200	583 A/T	No			NM 0251 NP 0794 NP 079		0.0195	0.0127	selective C		
U91581 U91581	305	220	2	26+08 SNV	C	G	No	Heterozys	45,000	100		0,472	280	645 C/G	No	PAX5	paired bo PAX3	NM 001127366.2 c 1171-1				0,0719 Craniofac 8		
AJ9158	1294	222	2	26408 SNV		G	No			195		0,4897	378	380 G/G	No	PAXS	paired bo PAX3	NM 000438.5 ± 1297xC NM		0,1231	0,1034	Craniofac B		
AJ91581	105	223	2	2E+08 SNV	Ĝ	A	No		51,667	99	84	0,459	183	361 A/G	No	MLPH	melanopt MLPH	NM 001042467.2:c234G>						1/13, 1/1
U91584	211	224	2	2E+08 SNV	T	c	No		48.276	136		0.4494	247	516 C/T	No	MLPH	melanopt MLPH	NM 001(NP 0770 NP 077						6/14.5/1
AJ91581	211	225	2	25+08 SNV	G	A	No	Heterozya	52,149	151		0.4757	288	549 A/G	No	MUPH	melanopt MLPH	NM 001(NP 0012 NP 077						6/14.5/
AJ91581	104	226	2	28+08 SNV	c	т	No	Heterozys	48,997	156		0,4925	268	550 C/T	No	MUPH	melanoph MLPH	NM_001042467.2:c.681C+T						6/13,7/1
AJ91581	210	227	2	28+08 SNV	с	т	No	Heterozys	46,108	107	126	0,4592	233	511 C/T	No	MUPH	melanopit MLPH	NM_001(NP_0770 NP_077						6/13,7/1
4AJ9158	302	228	2	2E+OB SNV	A	G	No	Homozyg	100	156	139	0,4712	295	295 G/G	No	MLPH	melanopit MLPH	NM_001(NP_0770 NP_077	0 NM_024: Yes					9/16, 9/1
HAJ9158	184	229	2	2E+08 SNV	T	C	No		99,153	281	280	0,4991	561	564 C/C	No	MUPH		NM_001(NP_0012 NP_077						8/14, 8/1
1010100	112	220		16,09 CMM	-	0	No.	Hataroosa		137		0.492	150	554 077	N-	ALC: NO		NM 001042467.2 v 1053Tv						0/14 0/1

Identification of whole exon deletions / duplications copy number variations (CNV) analysis



CNV analysis

Interpretation of causality

Genet Med. 2015 May ; 17(5): 405-424. doi:10.1038/gim.2015.30.

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

Sue Richards [Chair, ACMG],

Benign sequence variants
 Likely benign sequence variants
 Sequence variants of uncertain significance
 Likely pathogenic sequence variants
 Pathogenic sequence variants

Result:

A) Identification of pathogenic variant/variants

e.g. two pathogenic variants in CAPN3 > confirmed diagnosis of limb girdle muscular dystrophy

B) Identification of variants of uncertain significance: e.g. variant in SCN4A

- genetic-clinical correlation
- segregation of variant in family
- type of inheritance

C) Only benign variants identified - diagnosis was not confirmed

- > pathogenic variant in unanalyzed gene, in noncoding region
- ≻ WES, WGS

Limitations of NGS:

- panel + WES: analysis of coding regions sequencing about 95-98% of selected regions
- occurrence of pseudogene, regions with high similarity: difficult non-specific mapping
- is not a suitable method for diseases associated with the expansion / deletion of repetitive sequences

Molecular genetic diagnostics of monogenic diseases

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- 4. RP-PCR detection of repeat expansions
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Material: DNA isolated from the whole blood

- gold standard for CNV detection (whole exons deletions/duplications)
- targeted analysis of a specific gene/genes
- available for certain genes

MLPA probe gold standard for CNV detection • Binding site RPO reverse PCR primer targeted analysis of a specific gene/genes LPO • Binding site forward PCR primer Stuffer sequence available for certain genes • Hybridisation sequence Hybridisation sequence Probe target Sample DNA Method description: PROBE 1. Sample denaturation and probe hybridisation 3. Probe amplification 5. Data analysis Reverse PCR primer Provide Forward PCR primer Probe B (299 nt) Probe A (170 nt) -----COFFALYSER 2. Probe ligation 4. Fragment separation Probe B Probe A (299 nt)

First example:

Duchenne muscular dystrophy, gene *DMD* (chromosome X):

- whole exon deletions (68%) and duplications (10%)
- first choice method



200

1500

1000

500

1E



D2





Second example: Spinal muscular atrophy (SMA), gene SMN1

- autosomal recessive disease
- incidence: 1 in 6,000 10,000 live births
- second most frequent fatal disease with autosomal recessive inheritance (after cystic fibrosis)
- characterized by degeneration of alpha motor neurons
- newborn screening



- > 95% caused by homozygous deletion of the SMN1 gene
- SMN1 has its almost identical copy SMN2 gene
 (SMN1 and SMN2 are homologous to except for few nucleotides)
- copy number variation of SMN1 and SMN2 in human genome



Second example:

Spinal muscular atrophy (SMA), SMN1

- > patients with homozygous *SMN1* deletion
- heterozygous carriers of SMN1 deletion
- SMN2 copy number

Control: 2x SMN1, 2x SMN2

SMA: 0x SMN1, 3x SMN2

SMA: 0x SMN1, 5x SMN2



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Material: DNA isolated from the whole blood

4. RepeatPrimed-PCR (RP-PCR)

> Method description:

- three primers
- characteristic profile of amplicons of increasing length, which differ by the length of a repeat unit (3 bp)



V. Mootha, Inv. ophthal. & vis. science 2014

- detection of repeat expansions (usually three nucleotides)
- presence / absence of expansion
- does not determine length of expansion (number of repeats)

RP-PCR and fragment analysis:





Myotonic dystrophy type 1

> expansion of CTG repeat in 3'UTR of DMPK (9q13.32)

- > autosomal dominant inheritance
- correlation between number of repeats and severity of the phenotype:
 - 5-37 repeats unaffected
 - 38–50 repeats premutation, asymptomatic
 - 51–149 repeats mild adult-onset form
 - 150–1000 repeats classic MD1
 - >1000 repeats congenital form MD1

anticipation - the number of repeats tends to increase in size over generations. Expansion of the CTG repeats commonly occurs during meiosis. As a result, children of affected individuals tend to have severe symptoms and earlier onset than their parents.

20x CTG

tccgcggccg gcgaacgggg ctcgaagggt ccttgtagcc gggaatg<mark>ctg ctgctgctgc tgctgctgc gctgctgctg ctgctgctgc tgctgctgct gctgctgggg ggatcacaga ccatttcttt ctttcggcca ggctgaggcc ctgacgtgga tgggcaaact gcaggcctgg</mark>

140x CTG

tccgcggccg gcgaacgggg ctcgaagggt ccttgtagcc gggaatgctg ctgctgctgct tgctgctgct gctgctgct ctgctgctgc tgctgctgct gctgctgct ctgctgctgct tgctgctgct gctgctgctg ctgctgctgc tgctgctgct gctgctgctg ctgctgctgct tgctgctgct gctgctgctg ctgctgctgc tgctgctgct gctgctgctg ctgctgctgct tgctgctgct gctgctgctg ctgctgctgc tgctgctgct gctgctgctg ctgctgctgc tgctgctgct gctgctgctg ctgctgctgc tgctgctgct gctgctgggg ggatcacaga ccatttcttt cttcggcca ggctgaggcc ctgacgtga tgggcaaact gcaggcctgg

Myotonic dystrophy type 1 – mechanism:

- toxic effect of expansion
- accumulation of RNA with expansions in the nucleus, sequestration of RNA-binding protein > formation of nuclear inclusions
- altering mRNA splicing of other genes





Mignon, IONIS-DMPK Clinical Program in Myotonic Dystrophy



Image shows the location of the Mbnl1 splicing factor (green) and the second image shows the location of RNA repeats (red) inside the cell nucleus (blue). The white arrows point to two large foci in the cell nucleus where Mbnl1 is sequestered with RNA. Photos by Hongqing Du

Turner, Journal of Neurology, Neurosurgery & Psychiatry 2010

Myotonic dystrophy type 1 – result:

- presence / absence of expansion
- not the length



Solution: Southern blot and hybridization

RP-PCR and fragment analysis:





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Material: DNA isolated from the whole blood

4. Southern blot and hybridization

detection of repeat expansions / deletions \succ determination of the size \geq

bp

Method description:

- DNA is cleaved by a restriction endonuclease ٠
- electrophoresis ٠
- transfer to membrane •
- hybridization with radioactive labeled probe ٠
- autoradiography ٠





MD1: 1 – negative control 2-5 – expansion

4. Southern blot and hybridization

Example:

Facioscapulohumeral dystrophy 1 (FSHD1)

- the third most prevalent muscular dystrophy, AD inheritance
- weakness and wasting of the face, shoulder and upper arm muscles, with later involvement of the trunk and lower extremities
- FSHD develops through complex genetic and epigenetic events that converge on a common mechanism of toxicity with mis-expression of the transcription factor DUX4



4. Southern blot and hybridization

Example: Facioscapulohumeral dystrophy 1 (FSHD1)

- 4q35, repeats D4Z4 (contain DUX4)
- 11-100 repeats \rightarrow unaffected
- 1-10 repeats \rightarrow affected



we determine the number of D4Z4 repeats according to the size of the product

Molecular genetic diagnostics:

- the results must be interpreted with knowledge of the molecular nature of the disease and knowledge of the structure and function of encoded protein
- the results must be interpreted in relation to the patient 's phenotype and results of other patient examinations (biochemistry, pathology, NMR, EMG, etc.)
- it is necessary to return to the results of already examined patients with an unconfirmed genetic diagnosis and test them with new techniques and perform new interpretations of the identified sequence variants
- it is necessary to participate in international quality control of DNA diagnostics for individual diseases

Example of specific treatment - certain disease, certain mutation

Spinal muscular atrophy (SMA)

Spinal muscular atrophy (SMA)

- gene SMN1, autosomal recessive disease
- incidence: 1 in 6,000 10,000 live births
- second most frequent fatal disease with autosomal recessive inheritance
- characterized by degeneration of alpha motor neurons
- newborn screening started this year

- > 95% caused by homozygous deletion of the SMN1 gene
- SMN1 has its almost identical copy SMN2 gene (SMN1 and SMN2 are homologous to except for few nucleotides)
- copy number variation of SMN1 and SMN2 in human genome



Clinical severity is modified by copy number the SMN2 gene



Spinal muscular atrophy

4 clinical types of SMA



Spinal muscular atrophy

Motoneuron degeneration in SMA



- functional degeneration of central synapses and neuromuscular junctions and subsequent axonal damage > motoneuron loss
- complete loss of motoneuron is a irreversible change
- The beneficial effects of SMA therapies are dependent on disease duration at the time of intervention. Disease duration before treatment is critical and a delayed intervention leads to a less efficient rescue. The effect of SMA therapies is strongest in pre-symptomatic patients.

SMA therapies

- 1. modifying splicing of SMN2 (production of more amount of full length mRNA)
- 2. replacing the SMN1 gene



1. modifying splicing of *SMN2* (production of more amount of full length mRNA)

A. Nusinersen (Spinraza®)

- an antisense-oligonucleotide (ASO) that enhances the inclusion of exon 7 in mRNA transcripts of SMN2
- administered intrathecally
- B. Risdaplam (Evrysdi®)
 - administered orally

2. replacing the SMN1 gene

C. Onasemnogene Abeparvovec-xioi (Zolgensma®)

- children younger than two
- one-time intravenous infusion
- adeno-associated virus 9 (AAV9) delivering cDNA which codes the full length SMN protein
- = replacement of a missing or faulty *SMN1* gene with a functioning gene



SMA neonatal screening pilot project in Czech republic

- > early detection of neonates in the preclinical asymptomatic stage
- > treatment before irreversible complete loss of motoneuron

QUESTIONS?