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Klinika dětské neurologie,
MU a FN Brno

PARENT PROJECT
svalová dystrofie
Výzkum Léčba Naděje


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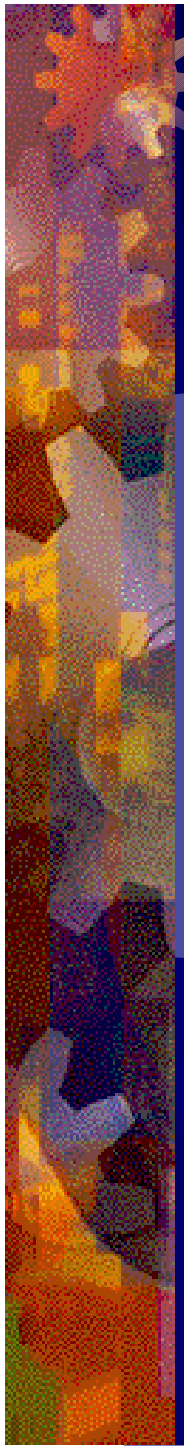
Almancil | Algarve | Portugal



Světový kongres s hlavním tématem: Léčba dědičných nervosvalových chorob, zejm. DMD/BMD a SMA

- Aktivní účast za finanční podpory Parent Projectu

1) Naše aktivní účast

- Posterová prezentace
- „Spectrum of point mutations in Czech DMD/BMD patients and their phenotypic outcome“

FAKULTNÍ NEMOCNICE BRNO

Spectrum of point mutations in Czech DMD/BMD patients and their phenotypic outcome.

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Introduction:

Duchenne and Becker muscular dystrophies (DMD/BMD) are X-linked recessive neuromuscular diseases caused by a mutation in the *DMD* gene. 60% of DMD/BMD cases are caused by
deletions, 5% by large duplications, and 35% by point mutations. DMD is typically connected with mutations causing a premature termination codon (PTC), while BMD with in-frame dele

Methods:

- Mutation screening on mRNA level: reverse transcription-PCR, protein truncation test, DNA sequencing.
- Mutation screening on DNA level: PCR-sequencing.
- Muscle biopsy + immunohistochemistry: dystrophin (DYS1, 2, 3), N-utrophin

Czech DMD/BMD patients with the mutation in the *DMD* gene

Patient	Phenotype	Localization of mutation (exon)	Mutation identified on mRNA level	Mutation identified on DNA level	Mutation at protein level	Immunohistochemical labelling using antibodies DYS1,2,3
1.	**BMD**	**3**	**c.[163G>T; 94_649del]**	**not performed**	**p.[Glu55X; F31_D217del]**	**not performed**
2.	**BMD**	**intron 3**	**c.187_364del**	**c.187-13_194del+21**	**p.Pro63_Asn88del**	**decreased intensity of DYS1 and DYS3**
3.	**DMD**	**intron 4**	**c.264_265ins265-395_265-464**	**c.265-463A>G**	**p.Val89MetfsX10**	**negative**
4.	DMD	7	c.583C>T	not performed	p.Arg195X	negative
5.	DMD	7	c.583C>T	not performed	p.Arg195X	negative
6.	DMD	intron 7	c.531_649del	c.649+5G>A	p.Pro178CysfsX2	DYS2 and DYS3 negative, DYS1 present at 20% of muscle fibres
7.	DMD	intron 7	not performed	c.649+5G>A	p.Pro178CysfsX2	not performed
8.	DMD	8	c.721C>T	not performed	p.Glu241X	not performed
9.	**DMD**	**9**	**c.835G>T**	**c.835G>T**	**p.Gly285X**	**negative**
10.	DMD	10	c.1062G>A	not performed	p.Trp354X	negative
11.	**DMD**	**11**	**c.1324C>T**	**not performed**	**p.Gln442X**	**negative**
12.	**BMD**	**intron 12**	**c.1483_1602del**	**c.1483-8T>G**	**p.Val495_Lys534del**	**DYS1 negative, DYS2 and DYS3 decreased intensity**
13.	DMD	16	c.1873C>T	not performed	p.Gln625X	negative
14.	DMD	16	c.1961T>C	c.1961T>C	p.Leu654X	not performed
15.	**DMD**	**18**	**c.2276T>G**	**not performed**	**p.Leu759X**	**negative**
16.	DMD	18	c.2281G>T	not performed	p.Glu761X	negative
17.	**DMD**	**20**	**not performed**	**c.2566C>T**	**p.Gln856X**	**not performed**
18.	**DMD**	**intron 20**	**c.2622_2623insAG**	**c.2623-3C>G**	**p.Asp875ArgfsX17**	**negative**
19.	DMD	21	c.2795C>T	not performed	p.Ser933X	negative
20.	DMD	24	c.[=, 3165_3276del]	c.3276+1G>A	p.Asn1055_Arg1092del	decreased intensity
21.	**BMD**	**25**	**c.[3328G>T; 3277_3432del]**	**not performed**	**p.[Glu1110X; Leu1093_Gln1144del]**	**decreased intensity**
22.	**BMD**	**25**	**c.3277_3432del**	**c.3432G>A**	**p.Leu1093_Gln1144del**	**DYS2 and DYS2 normal, DYS3 decreased intensity**
23.	BMD	intron 25	c.[=, 3277_3432del]	c.3433+1G>A	p.[=, Leu1093_Gln1144del]	normal
24.	**BMD**	**intron 25**	**c.[=, 3277_3433del]**	**c.3432+1T>C**	**p.[=, Leu1093_Gln1144del]**	**DYS3 negative, DYS1 and DYS2 decreased intensity**
25.	DMD + CMT1A	27	**c.3609_3611del TAAinsCTT**	**not performed**	**p.Lys1204LeufsX11**	**almost negative**
26.	DMD	28	c. [3822C>G; 3787_3931del; 3787_4701del]	c.3822C>G	p.[Tyr1274X; Glu1263_Asp1307del; Glu1263_Glu1357del]	normal
27.	DMD	29	not performed	c.3982C>T	p.Gln1328X	not performed
28.	**DMD**	**30**	**c.4099C>T**	**not performed**	**p.Gln1367X**	**negative**
29.	DMD	33	**c.4638delA**	**not performed**	**p.Ala1547LeufsX2**	**negative**
30.	**BMD**	**intron 33**	**C[=, 4674_4675ins10]**	**c.4675-11A>G**	**p.[=, Val1559PhefsX28]**	**DYS1 negative, Dys2 and DYS3 decreased intensity**
31.	DMD	34	c.4729C>T	not performed	p.Arg1577X	negative
32.	DMD	36	c.5031C>A	not performed	p.Tyr1677X	negative
33.	DMD	39	c.5434G>T	not performed	p.Glu1812X	negative
34.	DMD	39	c.5580delC	not performed	p.Glu1858AsnfsX13	negative
35.	DMD	40	c.5683C>T	not performed	p.Gln1895X	negative
36.	DMD	40	c.5662delC	not performed	Asp1888IlefsX3	negative
37.	DMD	42	c.5985T>G	not performed	c.5985T>G	negative

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Results:

- Excluding deletions and duplications by MLPA (Multiplex Ligation dependent Prob Amplification), 65 different point mutations in 63 DMD/BMD patients and 5 DMD fe carriers were identified. 38 types of mutations were unique for Czech DMD/BMD pop (mentioned by bold letters in the Table).
- 51 patients have DMD phenotype, 12 patients BMD phenotype.
- Two patients (3 and 55) have point mutations localised deep in introns. These mutatio create new splicing signals and cause insertions of a part of intron sequence into mRNA
- One patient (49) carries an insertion of the repetitive sequence *AluYa5*. This insertion disrupted the splicing signal and led to the frame-shift deletion.
- Four patients have mutations creating PTC and BMD phenotype (patients 1, 21, 26 and All of these cases can be explained by molecular mechanisms connected with expressio genetic information (dystrophin production is initiated at the new start codon, mutations change an splicing enhancer sequence and cause in-frame deletions, mutations near the 3´end can escape from no mediated mRNA decay).

Cases with interesting genotype-phenotype correlations:

Patient 21 – the mutation c.3328G>T, p.Glu1110X; BMD phenotype – the mutation changes an exon splicing enhancer sequence in exon 25 and causes in-frame deletion of exon 25 (predicted by Rescue-ESE program, *http://genes.mit.edu/burgelab/rescue-ese/*).

Significantly decreased intensity of dystrophin immunostai patient 21 compared with normal control (immunohistoche DYS2 (C-terminal antibody), original magnification x200).

Reverse transcription-PCR in patient 21 (P) shows transcript with the mutation p.Glu1110X and transcript with the in-frame deletion of exon 25, compared with control sample (C).

Patient 22 – the mutation c.3432G>A; BMD phenotype – the mutation does not change the codon p.Q1144 but changes splicing of exon 25 and generates mRNA with the in-frame deletion of exon 25.

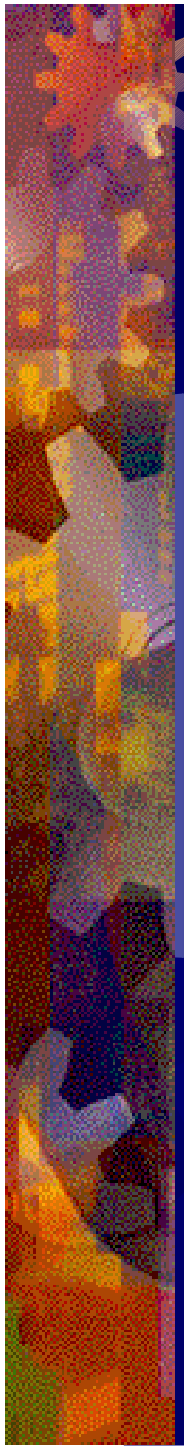
tcatatctaatatgtggcagtaatttttttcagctggcttaaattgatttatttttctt
TTAGTCAGTGATATTCAGACAATTCAGCCCAGTCTAAACAGTGTCAATGAAGGTGGCA
ATAAAGAATGAAGCAGAGCCAGAGTTGCTTCGACACTTGAGACAGAACTCAAAGAACT
ACTCAGTGGGATCACATGTGCCAACAG**gt**atagacaatctctttcactgtggcttgcctc
gtacttaactaaagatttcctaatgtactgagttcttacacaagatcaag

Point mutations in exon 25 (red letters) causing exon skipping in patient 21 and patient 22 (exon sequence is mentioned by capital letters, intron conserved sequences by green letters).

Insertions of parts introns into mRNA caused by single nucleotide changes deep in introns:

Patient 3 – the mutation c.265-463A>G caused creation of a novel splice site, and the insertion of

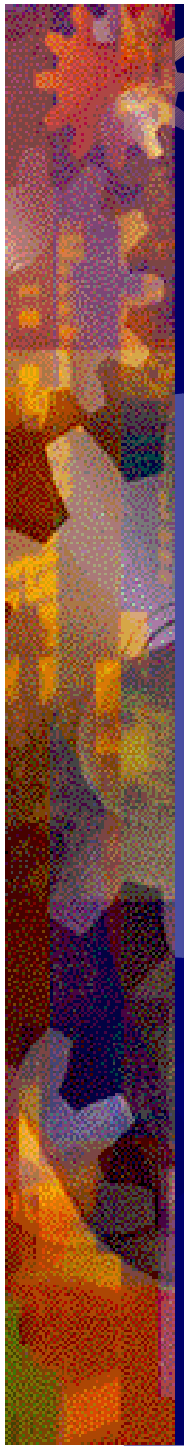
Patient 55 – the mutation c.9564-427T>G caused creation of a novel splice site, and insertion of 51



Práce prezentuje bodové mutace v DMD genu u všech 63 pacientů a 5 přenašeček DMD/BMD na podkladě bodových mutací, kteří byli dosud diagnostikováni v ČR

® Význam pro budoucí molekulárně genetickou terapii DMD/BMD

® Práce živě diskutována a velmi oceněna zejm. holandskými specialisty na problematiku DMD/BMD



2) Zhodnocení všech ve světě aktuálně probíhajících a plánovaných klinických studií

- Exon skipping
- Inhibitory myostatinu
- Genová terapie s virovými vektory
- Kmenové buňky

3) Horké novinky

- Kromě pokračujícího testování exon skippingu 51 (III. fáze) a 44 (I/II. fáze), již od 2012 klinické testování skippingu dalších exonů:
  45 a 53 (1. polovina 2012)
  52 a 55 (preklinický vývoj)

- Rozšíření spektra delecí + již také duplikací, které bude možno léčit exon skippingem

- **Kombinace exon skippingu a inhibice myostatinu (duální exon skipping v obou genech současně)**
- **Kombinace kmenových buněk a transferu minidystrofinu virovými vektory (buněčná a genová terapie)**

4) Osobní kontakty

- diskuse s hlavními koordinátory klinických studií ve světě a výměna informací o naší studii DMD114044 a dalším společně plánovaném a koordinovaném postupu při testování terapie DMD/BMD
  - Natalie Goemans (Leuven, Belgie)
  - Thomas Voit (Paříž, Francie)
  - Kate Bushby (Newcastle, UK)
  - Kevin Flanagan (Columbus, Ohio, USA)

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