Differential diagnosis of Duchenne/Becker muscular dystrophy by molecular analyses in Hungary

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Workshop on international care standards for DMD patients, Budapest, 18. April, 2012.
Duchenne/Becker Muscular Dystrophy

- DMD/BMD caused by mutation of the dystrophin gene; XR
- DMD incidence of 1 in 3500 live born males
- BMD is a milder form with a later onset and slower clinical progression; incidence 1:30000
**Genetic background**

The dystrophin gene (Xp21) has 79 coding exons, substantial amount of alternative splicing and at least seven tissue-specific promoters.

**Mutations**
- Deletions (60 %), in hot-spot regions of the gene (ex02- ex10 and ex44-ex52)
- Intragenic duplications (5-8 %)
- Point mutations and splicing errors (30-35 %)

1/3 of cases are de novo mutations.

High ratio of germinal/somatic mosaicism.

**Phenotype and genotype correlations**

- **In frame mutation:** Becker phenotype (reduced dystrophin protein level or truncated dystrophin)
- **Out of frame mutation:** Duchenne phenotype (absence of dystrophin protein)

Point mutations mostly with stop codons – DMD phenotype.
Molecular analyses of the dystrophin gene and of the protein

- **Multiplex PCR reaction** (2x9 exons simultaneously amplified in two reactions; Beggs and Chamberlain), since 2001.
- **Southern blot** using cDNA probes (XJ10, 7b8, 30.2, 30.1, 47.4, 60.1) since 2002.
- **MLPA**: Multiplex Ligation-dependent Probe Amplification (79 exons and promoter analysed; MRC Holland), since 2006.
- Immunohistochemistry of muscle biopsy (LMU Munich, H. Lochmüller, Molnar MJ, SE Clinic for Neurology)
- Western blot technique (LMU Munich, H. Lochmüller, Molnar MJ, SE Clinic for Neurology)
- Sequencing of the dystrophin gene (Univ. Würzburg, Leiden, Ferrara)
Protein analysis in dystrophinopathies

I. Immunohistochemistry

II. Immunobloting (Western blot)

Control   DMD patient

DMD carrier female   BMD patient

Kaplan et al. 2002
Multiplex PCR analysis
MLPA analysis

Dystrophin gene ex45-ex46 deletion

Dystrophin gene ex51-ex55 duplication
NMD-CHIP array analysis in the dystrophin gene

Sample 66/3, affected boy, deletion Dp427m-ex44 (Score:-0.715)
NMD-CHIP array analysis in the dystrophin gene

Sample 66/2, carrier mother, deletion Dp427m-ex44 (Score: -0.281)
NMD-CHIP array analysis in the dystrophin gene

Sample 195/3 affected boy, duplication ex44 and ex48-ex55 (Score:+0.346)
NMD-CHIP array analysis in the dystrophin gene

Sample 195/2 carrier mother, duplication ex44 és ex48-55 (Score:+0.315)
NMD-CHIP array analysis in the dystrophin gene

Sample 97/2 manifest carrier female, deletion in ex10-ex44 (Score:-0.385)
Results - male patients

318 DMD/BMD male patients analysed

- In 119 patients no mutations found
- In 199 patients mutations confirmed
  - In 23 patients duplications found
  - In 163 patients deletions found
    - In 149 patients deletions in hot-spot regions (ex44-ex52 and ex02-ex10)
    - In 12 patients rare deletions (detected only by MLPA)
  - In 13 patients point mutations confirmed
  - 2 patients with contiguous gene deletion syndrome (dystrophin + ARX, IL1RAP1l, NR0B1, GK, RPGR genes)
Results – female relatives, prenatal diagnosis

150 female relatives analysed

15 males out of 40 prenatal cases

5 unaffected
10 affected

105 mothers

48 non-carrier mothers found
57 carrier mothers found

5 daughters

5 manifesting carriers (2 de novo cases)

40 sisters/cousins

17 non-carrier sisters/cousins found
11 carrier sisters/cousins found

12 cases confirmed by MLPA without having the sample of index patient