Patterns of Deletions and the Distribution of Breakpoints in the Dystrophin Gene in Czech Patients with Duchenne and Becker Muscular Dystrophy (Statistical Comparison with Results from Several Other Countries)

(Duchenne and Becker muscular dystrophy / dystrophin gene / deletions / breakpoints / comparison among populations)

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Abstract. Deletion pattern analysis of the dystrophin gene was performed in 115 unrelated Czech patients with Duchenne and Becker muscular dystrophy. In 50 patients (43.5% of the analysed patients) exon deletions were detected by routinely performed multiplex PCR for 18 selected exons and for the area of muscle-specific promoter of the dystrophin gene. All start-points and endpoints of deletions (100 breakpoints) were detected using PCRs for another 29 exon areas of the dystrophin gene (altogether primers for 47 different exons were used). Most of the breakpoints were found in introns 44 (16% of breakpoints), 47 (14%) and 50 (8%). The comparison of distributions of breakpoints in the area of the main hot spot of the dystrophin gene (introns 43–52) was made (χ^2 test in a contingency table) in six different populations from the Czech Republic, Bulgaia, Hungary, Italy, Turkey and India. In compared populations, statistically significant differences were found by the pooled test. No significant difference between the Czech population and other studied populations was found by pair comparisons. On the other hand, pair comparisons revealed significant differences between populations from Bulgaria and Hungary, Bulgaria and Turkey, Hungary and Italy. The results of the presented study support the theory suggested by other authors that specific differences in intron sequences of the dystrophin gene can exist between different populations, possibly as a result of a genetic drift.

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Abbreviations: cDNA – complementary deoxyribonucleic acid, D/BMD – Duchenne and Becker muscular dystrophies, PCR – polymerase chain reaction.

Duchenne and Becker muscular dystrophies (D/BMD) are allelic, X-linked, recessive, neuromuscular disorders which are caused by mutations in the dystrophin gene (Emery, 1993). The gene, localized in the Xp21 locus, is 2.4 megabases long and has 79 exons (Koenig et al., 1987; Roberts et al., 1993). Although dystrophin mutations are heterogeneous, 60–65% of all mutations are deletions of one or more exons (Koenig et al., 1987; Strachan and Read, 1999). Almost regardless of the size of the deletion, frameshifting deletions produce lethal DMD, whereas non-frameshifting mutations cause the milder Becker form (Amalfitano et al., 1997; Strachan and Read, 1999). Gross deletions are localized in two hot spots in the gene. One hot spot is in the area of exons 1 to 20, and the second in the area of exons 45 to 55 (Koenig et al., 1987; Cooke et al., 1990). Almost all these large deletions (± 98%) can be detected by multiplex polymerase chain reactions (PCR) containing primers for 18 exons and the muscle-specific promoter of the dystrophin gene (Beggs et al., 1990; Chamberlain et al., 1990; Abbs et al., 1991). In studies that investigated the extents of deletions and the distributions of breakpoints in different populations, primers for other exons and/or cDNA probes were generally used (Vitiello et al., 1992; Danieli et al., 1993; Florentin et al., 1995; Todorova et al., 1996; Banerjee and Verma, 1997; Herczegfalvi et al., 1999; Önengüt et al., 2000). These studies suggest non-random location of some breakpoints in the dystrophin gene. Breakpoints in introns 44, 47 and 50 seem to be the most frequent in D/BMD patients from different populations. The ratio of the numbers of breakpoints between these introns can differ for different populations. Other introns with a higher number of breakpoints can be found as well, and they seem to be characteristic for different populations. The differences mentioned above can be due to diverse intronic sequences in different populations. These intragenic differences can be a result of a genetic drift

(Danieli et al., 1993; Önengüt et al., 2000) and could influence not only the distributions of breakpoints, but also the frequency of deletions in the dystrophin gene and thus even the incidence, respectively the prevalence of D/BMD in different populations. We report here the data on the dystrophin gene deletion pattern and the distribution of breakpoints obtained by screening Czech patients with D/BMD, and we also compare our findings with the findings of other authors.

Material and Methods

The DNA analysis for deletion screening of the dystrophin gene was performed in 115 unrelated Czech patients with clinical symptoms of D/BMD. The patients in study were considered to be a random selection from D/BMD patients in the Czech population. The DNA analysis was performed in the Thomayer University Hospital in Prague. Genomic DNA was extracted from peripheral blood lymphocytes by the protein salting-out method (Miller et al., 1988). Deletion screening in all 115 patients was performed by multiplex PCR for regions of the muscle-specific promoter and eighteen selected exons of the dystrophin gene. The amplified exons were: 3, 4, 6, 8, 12, 13, 17, 19, 43, 44, 45, 47, 48, 49, 50, 51, 52 and 60. These multiplex tests were run using the primers and conditions published by Chamberlain et al. (1990) and Beggs et al. (1990). The deletions were detected in 50 of the examined patients. In order to find the startpoints and endpoints of all deletions we used other primers and conditions published by Covone et al. (1992) for exons 20, 22; Prior et al. (1995a) for exons 7, 9, 53; Prior et al. (1995b) for exon 54. The analysis was extended to include another 23 pairs of primers, which we created according to intron and exon sequences of the dystrophin gene published on the Leiden Muscular Dystrophy pages (http://www.dmd.nl/seqes/dmd_ex_seqes.html). The sequences of the primers were created as follows:

5′-CTA CAg AAT CCT ggC ATC AgT TAC-3′ / 5′-TTg TAC TTA CAA CAg TCC TCT AC-3′(exon 1); 5′-CAC TAA CAC ATC ATA ATg gAA AgT-3′ / 5′-AAg ATA CAC Agg TAC ATA gTC CAT-3′(exon 2); 5′-TTA ACA ggT TgA TTT AgT g-3′ / 5′-CAT TCA TCA ggA TTC TTA CCT g-3′(exon 5); 5′-ATT Tgg AAg CTC CTg AAg ACA Ag-3′/ 5′-CTC ATg AgT ATg AAA CTg gTC-3′(exon 10); 5′-CAT gAT ggA TTT gAC AgC CCA TC-3′ / 5′-gCT TTg TTT TTC CAT gCT AgC-3′(exon 11); 5′-TTg ATT gTC TCT TCT CCA gg-3′ / 5′-TCT CAC ACA TgA CAC ACC Tg-3′(exon 14); 5′-ggA ATT CTT TAA TgT CTT gCA gTg-3′ / 5′-gAA AgC Tag AAA gTA CAT ACg g-3′(exon 15); 5′-gAT CTT TCT TgT TTT AAC Agg-3′ / 5′-gAT AAT Tgg TAT CAC TAA CCT g-3′(exon 16); 5′-CTA TTA TTT TTT gCT gTC TTA gg-3′/ 5′-TTA ATg CAT AAC CTA CAT Tg-3′(exon 18); 5′-CAg gAT gAA gTC AAC Cgg CTA TC-3′ / 5′-ACT TAC TTg TCT gTA gCT CTT TC-3′(exon 21); 5′-CTT AAA TTg ATT TAT TTT CTT AgC-3′ / 5′-gTg AAA gAg ATT gTC TAT ACC Tg-3′(exon 25); 5′-TgT TTg TTT TgT ggA Agg TC-3′ / 5′-TTT ACC TTC ATC TCT TCA AC-3′(exon 26); 5′-gAg CAT TCT TTA TTT TTC AgA g-3′ / 5′-gAC CAA gAA AAg CAA CTg ACT TC-3′(exon 27); 5′-gTg ATA TAT ATT TCT TTC TTA gg-3′ / 5′-ggC TTA ATT TAC AAC TTA CAT C-3′(exon 28); 5′-CTC TTA TTC CTT CTT TTT Agg C-3′/ 5′-gAT TCC CAg ATg TAC TTg CCT g-3′(exon 30); 5′-ATg CTC TCC TTT TCA CAg gC-3′ / 5′-TTC TAg CCT TTT CTC TTA CC-3′(exon 35); 5′-CTT gCT CAT ggA ATA TAg Cg-3′ / 5′-gTA gAT CTT CCT ACC TTT CC-3′(exon 37); 5′-TTT Agg CCT CCA TTC CTT Tg-3′ / 5′-gTT CAT TCA CAA CCA ATT TAC C-3′(exon 38); 5′-TTg TAT ATC TAT ACC AgC AC-3′/ 5′-ACC TTC AgA gAC TCC TCT Tg-3′(exon 42); 5′-gCT AgA AgA ACA AAA gAA TAT CTT gTC-3′ / 5′-gAC TTg CTC AAg CTT TTC TTT TAg-3′(exon 46 = only coding area); 5′-ATg AgT TCA CTA ggT gCA CCA TTC-3′ / 5′-gAg TgC TAA AgC ggA AAT gCC TgA-3′(exon 55); 5′-CTT TTC ATC TCA TTT CAC Agg C-3′ / 5′-gTT CCA CAT TCA ATT ACC TC-3′(exon 58); 5′-gTC ATA TTg CCA ATT TAg Ag-3′ / 5′-gTA AAg AAg TAg ACC gTA CC-3′(exon 59).

The reaction conditions for amplification (30 cycles) of the above mentioned 23 exons were as follows: denaturation temperature = 94°C/1 min; annealing temperature = 55°C (exon 5), 58°C (exons 5, 25, 30, 46, 56, 58), 60°C (exons 16, 39), 63°C (remaining exons)/1 min; elongation temperature 72°C/2.5 min. These PCR analyses were performed with approximately 100–200 ng genomic DNA; 0.8 U Taq polymerase (PCR buffer = 10 mM Tris HCl, 50 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$) (Top-Bio, s.r.o., Prague, Czech Republic); 0.25 mM dNTPs (Boehringer Mannheim GmbH, Mannheim, Germany); 8 pM of each primer (made by Life Technologies, Paisley, U.K.). The total volume of the PCR mixture was 25 µl. All the PCR products were detected on 2% agarose gel stained with ethidium bromide.

In this study, we altogether used primers for 47 different exons and for the muscle-specific promoter of the dystrophin gene.

We have compared the distributions of breakpoints in the area of introns 43–52 of the dystrophin gene by statistical analysis. We compared the data from the Czech population (present study) and data obtained by other authors from Bulgaria (Todorova et al., 1996), Hungary (Herczegfalvi et al., 1999), Italy (Vitiello et al., 1992), Turkey (Önengüt et al., 2000) and India (Banerjee and Verma, 1997). In the area of introns 43–52 all breakpoints were obtained in all compared populations (only one breakpoint was not obtained in the Bulgarian population). Statistical analysis was applied by means of the χ^2 test in a contingency table in order to detect statistically significant differences between frequencies of genetic patterns in different populations. The null hypothesis (the populations do not differ) was tested among all evaluated popu-