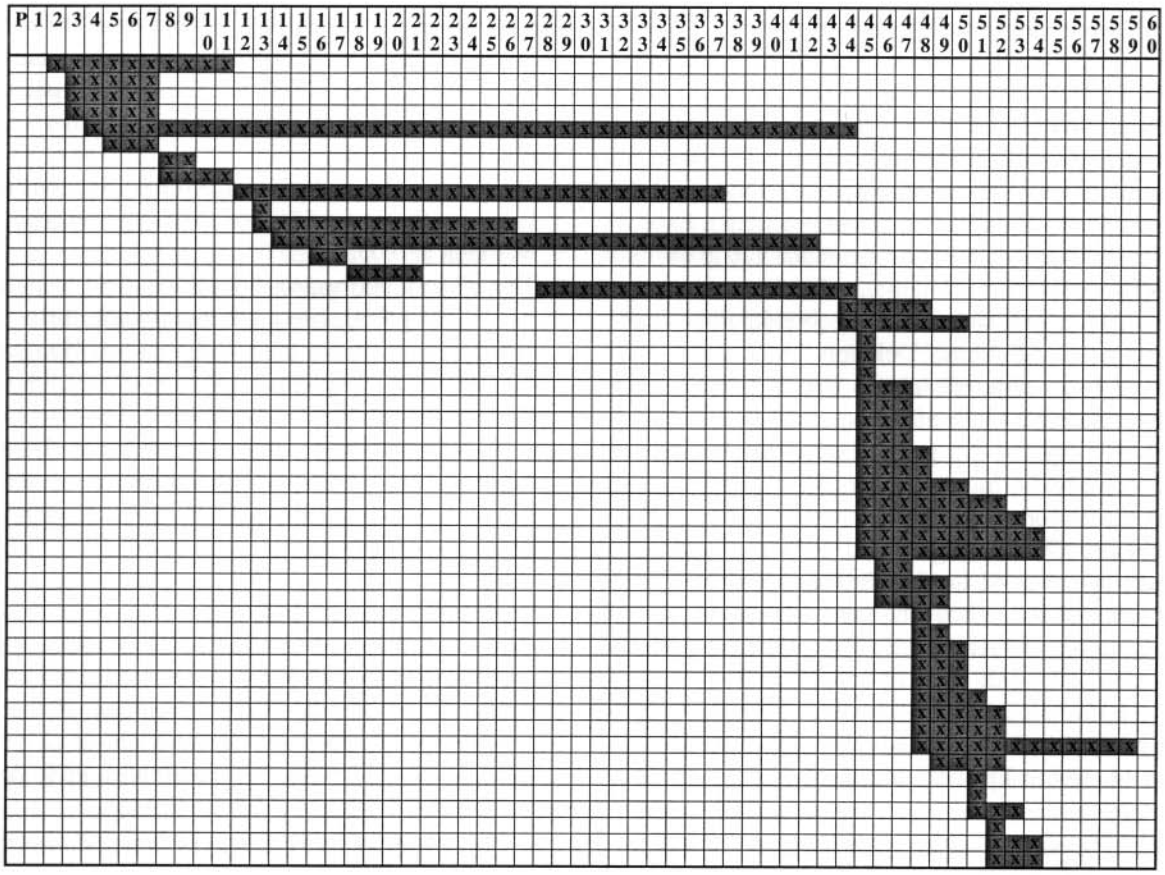
lations by the pooled test (P = 0.05) as well as by all possible pair comparisons (15 possible pairs) using Bonferroni correction (P = 0.05/15 = 0.0033).

Results

The analysis of deletions of the dystrophin gene was made in 115 unrelated Czech patients with clinical symptoms of D/BMD. Intragenic deletions were found in 50 patients (43.5% of the total number of patients). The localization and size of deletions in Czech patients are shown in Fig. 1. In 12 patients (24% of patients with deletion) we detected deletions in the first half (upstream of exon 40) of the gene. In 35 patients (70%) deletions were detected in the second half (downstream of exon 40) of the gene. Thus, the proximal/distal ratio of deletions was 1/2.92. In 3 patients (6%) the deletions



PFig. 1. Localization and size of deletions in the dystrophin gene observed in 50 unrelated Czech D/BMD patients. Top line: P = muscle-specific promoter; 1–60 = exons of the dystrophin gene. Lines 2–51: deletion patterns of selected D/BMD patients. ■ = deleted exon.

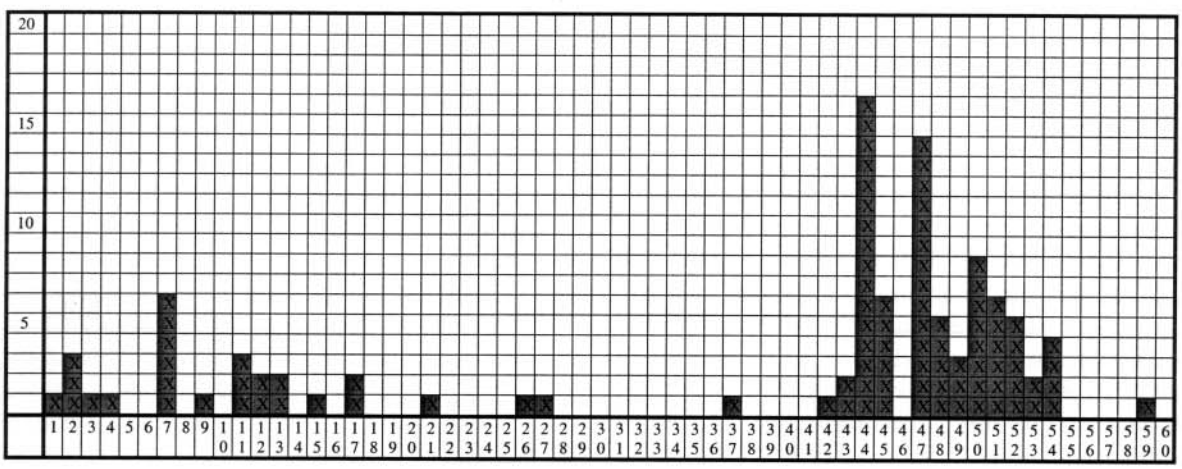


Fig. 2. Distribution of deletion breakpoints in the introns of the dystrophin gene observed in 50 unrelated Czech D/BMD patients. Left column: number of breakpoints = %. Bottom line: 1–60 = introns of the dystrophin gene. ■ = one breakpoint.

started in the first half of the gene and ended in the second half of the gene. The number of deleted exons was different in individual patients. The deletions affecting 3 consecutive exons (11 patients, i.e. 22%) were the most frequent. In 8 patients (16%) we detected deletions "only" in the area of one exon. The longest deletion found in our patient contained 41 exons and was located in the area of exons 4–44. The extents of deletions that we found are (expressed as the number of deleted exons/number of patients/percentage): 1/8/16; 2/4/8; 3/11/22; 4/8/16; 5/6/12; 6/1/2; 7/1/2; 8/1/2; 9/1/2; 10/3/6; 12/1/2; 14/1/2; 17/1/2; 26/1/2; 29/1/2; 41/1/2.

Thirty-five types of specific deletions were detected. Most of the patients had unique deletions (25 patients = 50% of deletions). The most frequent deletion was that of exons 45–47 (4 patients = 8% of deletions). Exon 48 was included in most of the deletions (20 patients = 40% of patients with deletions).

Altogether, we detected 100 breakpoints in 50 patients with deletions. Deletion breakpoints were observed in 28 different introns (see Fig. 2). Twenty-seven breakpoints (27% of breakpoints) were localized in the first half of the gene. Exactly 23 breakpoints (23%) were found in introns 1 to 20, and in introns 21 to 40 there were 4 breakpoints (4%). Seventy-three breakpoints (73%) were found in the second half (namely in introns 41 to 60). The following breakpoints were detected throughout the gene (shown as affected intron/number of breakpoints): 1/1; 2/3; 3/1; 4/1; 7/6; 9/1; 11/3; 12/2; 13/2; 15/1; 17/2; 21/1; 26/1; 37/1; 42/1; 43/2; 44/16; 45/6; 47/13; 48/6; 49/3; 50/8; 51/6; 52/5; 53/2; 54/4; 59/1. Most of the breakpoints were found in intron 44 (16 breakpoints). Intron 44 was also the most frequent startpoint of deletions (14 breaks). The most frequent endpoints of deletions were in introns 47 and 50 (5 breaks in each of them).

In statistical analyses of data from the Czech Republic (present study), 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) we tested the following null hypothesis: "The populations of the mentioned countries do not differ in the distribution of the number of deletion breakpoints in introns 43–52 of the dystrophin gene." The numbers of deletion breakpoints are shown in Table 1.

These were the findings:

1. The pooled χ^2 test showed that the populations differ significantly (P = 4.57E-006). The significance level was kept at 0.05.
2. The χ^2 test performed for all possible population pairs (15 pairs) revealed significant differences between populations from Bulgaria and Hungary (P = 1.98E-003), Bulgaria and Turkey (P = 9.30E-004), Hungary and Italy (P = 4.09E-005). The Czech population did not show significant differences from any other tested population. The significance level was kept at 0.05/15 = 0.0033. The P values of pair χ^2 tests are shown in Table 2.

Discussion

The DNA diagnostics of D/BMD is routinely performed by multiplex PCR for 18 selected exons and for the area of the muscle-specific promoter of the dystrophin gene described by Chamberlain et al. (1990) and Beggs et al. (1990). This multiplex PCR detects

Table 1. The numbers of deletion breakpoints in areas of introns 43–52 of the dystrophin gene in populations from the Czech Republic, Bulgaria, Hungary, Italia, Turkey and India

Intron:	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	***Total breakpoints:***
Czech Rep. *breakpoints:*	2	16	6	0	14	5	3	8	6	5	65
Bulgaria *breakpoints:*	4	14	4	0	10	12	3	17	12	8	84
Hungary *breakpoints:*	6	35	10	18	20	7	18	30	13	29	186
Italy *breakpoints:*	2	26	13	4	22	12	5	8	2	5	99
Turkey *breakpoints:*	12	59	15	12	31	23	7	38	12	21	230
India *breakpoints:*	3	32	9	9	22	16	21	31	11	8	162
Total breakpoints:	29	182	57	43	119	75	57	132	56	76	826

Table 2. P values of pair χ^2 tests in a contingency table[a]

	Czech Rep.	**Bulgaria**	**Hungary**	**Italy**	**Turkey**	**India**
Czech Rep.	–					
Bulgaria	3.96E–001	–				
Hungary	3.10E–002	1.98E–003	–			
Italy	4.13E–001	8.67E–002	4.09E–005	–		
Turkey	3.94E–001	9.30E–004	4.38E–003	5.88E–002	–	
India	1.73E–001	3.67E–002	2.91E–002	1.58E–002	1.49E–002	–

[a] P values lower than or equal to 0.0033 are underlined.