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Short insertions in the partner strands greatly enhance the discriminating power of DNA heteroduplex analysis: resolution of HLA-DQB1 polymorphisms

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Heteroduplex analysis has proved to be a good approach to the study of polymorphic systems (1–3) such as the HLA-DRB3, DPB1 and DRB1 genes (4–6). However, in the case of HLA-DQB1 locus, none of the alleles (differing from each other by one to 28 substitutions) gave a satisfactory resolution when used for providing partner strands in the generation of heteroduplex molecules. Hence, we modified two selected alleles by introducing three additional nucleotides. Their use as heteroduplex generators strikingly enhanced the resolving power of the technique.

The DQB1 alleles were first subdivided in two subsets by group-specific PCRs based on the 3′-end polymorphic region of DQB1 second exon. To this purpose, a 5′-end universal primer (DQBAMP-A) was used in combination with either of two 3′-end specific primers: DQB202 and DQB204 (7), respectively for the first group and the second group alleles (Fig. 1).

For each group, a 'reference' DNA was chosen: DQB1*0501 for the first group and DQB1*0302 for the second group. These two alleles were selected because they were the most discriminative following the analysis of all possible combinations. A 'reference' DNA common to both groups could not be used because of the extensive differences at the 3′-end region of the molecule that strongly disfavour the formation of heteroduplexes.

Both the DQB1*0501 and 0302 alleles were modified introducing, by a two-step procedure, a short insertion (TTT) in two non-polymorphic regions of the molecule at codons 42 or 62 (Fig 1). As an example, the insertion in position 42 in DQB1*0501 was obtained performing two simultaneous PCR reactions: one using the common primer DQAMP-A in combination with MT42as and the other using MT42s in combination with the group-specific primer DQB202. These PCR products were combined and used as templates in a following reaction carried out for the first five cycles in the absence of any primer, where strands elongation was driven only by the pairing of the terminal sequences carrying the insertion (i.e. the primers used in the first round of PCR). The reaction was then continued for other 27 cycles after the addition of the common 5′-end primer and the group-specific 3′-end primer DQB202 (Fig. 1). The final PCR products were cloned into pUC21 vector and sequenced.

The two modified 'reference' DNAs (5.1mut42, 3.2mut42, 5.1mut62 and 3.2mut62), amplified from the sequenced pUC21clones, were mixed with each allele of the same group, denatured by boiling, renatured at room temperature and run on a 12% polyacrylamide gel. The resolution obtained was remarkable: with the exception of DQB1*0401 and 0402 in group two, all the alleles became amenable to typing by one or the other of the two modified reference DNAs.

Figure 2A shows group 1 alleles mixed with the unmodified DQB1*0501 allele and with the same allele modified in position 42 and in position 62. The heteroduplexes formed with 5.1mut42 generated a distinct pattern for each allele, although 5.1mut62 gave better resolution in some cases (0501 from 0502, lanes 8 and 9 compared with lanes 15 and 16). Figure 2B shows the second group alleles mixed with the unmodified DQB1*0302 allele, with 3.2mut42 and with 3.2mut62. Some alleles were better distinguished by 3.2mut42 (0302 from 0303: lanes 9 and 10), while others were better resolved by 3.2mut62, (0301 from 0303, lanes 14 and 16, respectively). DQB1*0401 and 0402 could not be distinguished by either case. These two alleles differ by only one nucleotide near the 5′-end primer (T versus G at codon 23; see Fig. 1) and, in our experience, mismatches near the end of the molecule do not usually influence gel retardation. In more central positions, even single substitutions can be identified, as for the above mentioned 0302 and 0303 alleles, which differ for an A/C polymorphism in codon 57 (Fig. 1). Forty samples from our panel of oligotyped individuals were re-typed by this procedure, also in those cases where the two alleles fell within the same subgroup (an example for DQB1-group 2 typing is shown in Fig. 3). The results were in full agreement with the data obtained using oligonucleotide probes.

In conclusion, the insertion of short loop-forming sequences can greatly improve the resolution of complex as well as single base polymorphisms making the heteroduplex approach a more suitable technique for the analysis of genetic heterogeneity.

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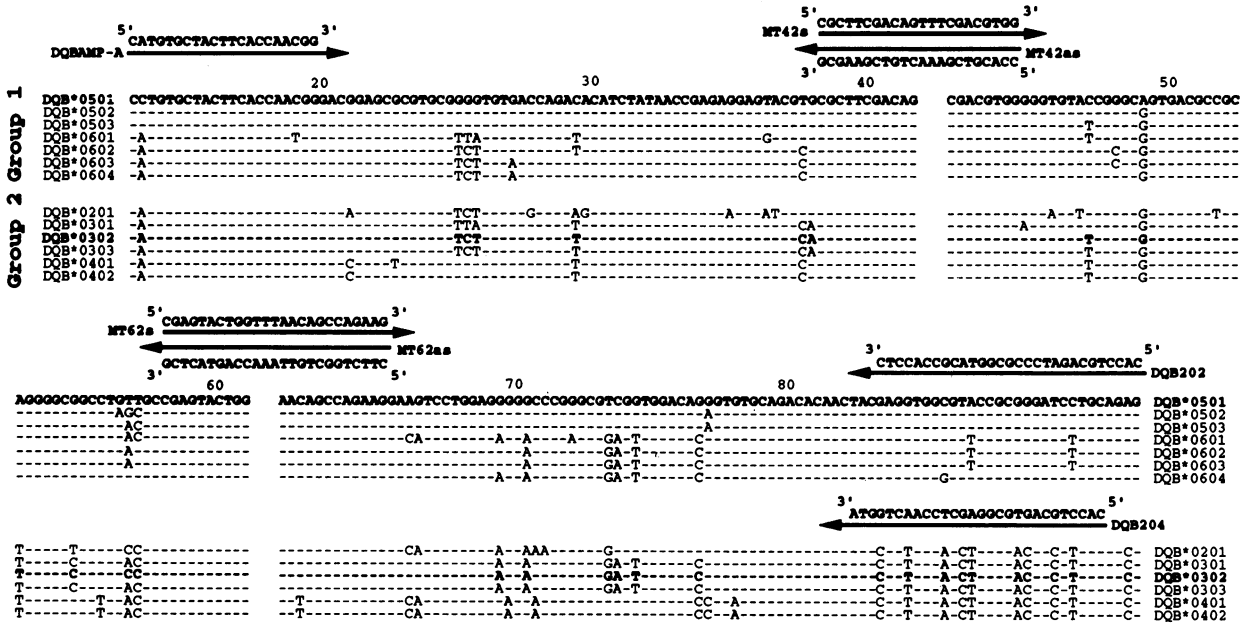

Figure 1. Alignement of nucleotide sequences of the PCR-amplified exon 2 region in the DQB1 alleles. Gaps in the sequences have been inserted to align the additional bases of the primers used for trinucleotide insertions. For group 1 and group 2 specific amplification, DQBAMP-A was used in combination with either DQB202 or DQB204 primers, respectively. Insertions were obtained on both DQB1*0501 and DQB1*0302 alleles (bold type) by using MT42s and MT42as for position 42 insertion and MT62s and MT62as for position 62 insertion. Antisense primers (MT42as and MT62as) were used in combination with DQBAMP-A on both alleles, while sense primers (MT42s and MT62s) were used in combination with either DQB202 or DQB204 for DQB1*0501 and DQB1*0302 alleles, respectively. PCR reactions were performed in all cases with 30 s steps of denaturation at 94°C, annealing at 62°C and extension at 72°C. Amino acid positions are reported with numbers above the DQB1*0501 consensus sequence.

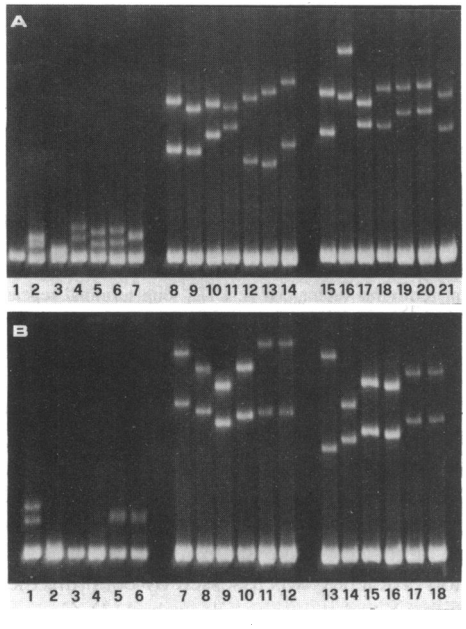

Figure 2. DNA heteroduplex patterns of DQB1 group 1 (Fig. 2A) and group 2 (Fig. 2B) alleles. All alleles have been amplified from HLA-typed homozygous cell lines and run on a 12% polyacrylamide gel over night at 15 mA in 1 × TAE buffer. (**A**) Group 1 alleles were mixed with unmodified DQB1*0501 (lanes 1–7), 5.1mut42 (lanes 8–14) and 5.1mut62 (lanes 15–21). Lanes 1, 8 and 15: DQB1*0501; lanes 2, 9 and 16: DQB1*0502; lanes: 3, 10 and 17: DQB1*0503; lanes 4, 11 and 18: DQB1*0601; lanes 5, 12 and 19: DQB1*0602; lanes 6, 13 and 20: DQB1*0603; lanes 7, 14 and 21: DQB1*0604. (**B**) Group 2 alleles were mixed with unmodified DQB1*0302 (lanes 1–6), 3.2mut42 (lanes 7–12) and 3.2mut62 (lanes 13–18). Lanes 1, 7 and 13: DQB1*0201; lanes 2, 8 and 14: DQB1*0301; lanes 3, 9 and 15: DQB1*0302; lanes 4, 10 and 16: DQB1*0303; lanes 5, 11 and 17: DQB1*0401; lanes 6, 12 and 18: DQB1*0402.

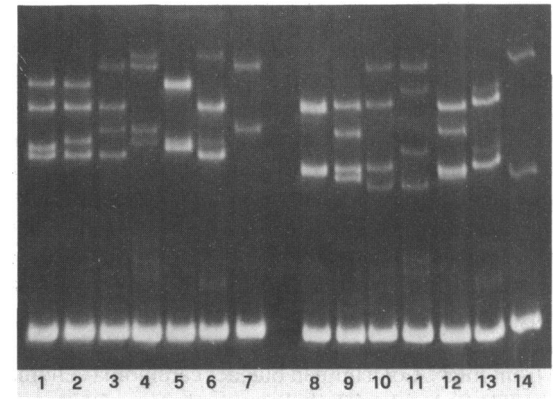

Figure 3. Group 2 HLA-DQB1 typing of random individuals. Seven DNA samples from our panel of oligotyped individuals were amplified with group 2 specific primers and mixed with 3.2mut42 (lanes 1–7) and with 3.2mut62 (lanes 8–14). The typing was as follows: lanes 1 and 8: DQB1*0302/*0303; lanes 2 and 9: DQB1*0301/*0302; lanes 3 and 10: DQB1*0302/*0201; lanes 4 and 11: DQB1*0201/*04; lanes 5 and 12: DQB1*0301/*0303; lanes 6 and 13: DQB1*0302/*04; lanes 7 and 14: DQB1*0201/*0201.

REFERENCES

1 Prior, T.W. *et al.* (1994) *Am. J. Med. Genet.*, **50**, 68–73.
2 Wang, Y.H. *et al.* (1992) *J. Biol. Chem.*, **267**, 4911–4915.
3 Delwart, E.L. *et al.* (1993) *Science*, **262**, 1257–1261.
4 Sorrentino, R. *et al.* (1991) *Immunogenetics*, **33**, 118–123.
5 Sorrentino, R. *et al.* (1992) *Immunogenetics*, **36**, 248–254.
6 D'Amato M. and Sorrentino R. (1994) *Tissue Antigens*, **43**, 295–301.
7 Nomura, N. *et al.* (1991)*Tissue Antigens*, **38**, 53–59.