shown that p73 was very frequently methylated in natural killer cell lymphomas.5 In lymphoma cell lines, methylation of p73 correlated with down-regulation of the p73 protein, and promoter demethylation led to re-expression of p73.5,7 Interestingly, our results also showed that p73 was frequently methylated in DLBC lymphoma in the stomach but not the lymph node. The significance of this in gastric lymphomagenesis merits further investigation.

Finally, the significance of gene methylation in MALT/MZBC and DLBC lymphomagenesis will need to studied by demonstrating that methylation-induced gene suppression contributes to cellular growth dysregulation or transformation.

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References

Rapid genotyping of Xbal and MspI DNA polymorphisms of the human factor VIII gene: estimation of their combined heterozygosity in the Argentinean population

In hemophilia A, indirect analysis using factor VIII gene polymorphisms is particularly valuable to obtain rapid information for genetic counseling. Herein, we describe an alternative route to investigate two intron 22 DNA polymorphisms (Xbal and MspI) using an intragenic 12 kb-long amplimer. The estimated heterozygosities on 37 haplotypes from the Argentinean population were Xbal (49%), MspI (50%), and combined Xbal+MspI (63%).

Hemophilia A (HA) is an X-linked inherited bleeding disorder due to deficiency in the coagulation factor VIII (FVIII).
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Letters to the Editor

Except for intron 22 (Inv22) and intron 1 inversions, responsible for approximately one half of severe phenotypes, direct HA mutation diagnosis is time-consuming and labor-intensive because of the size and complexity of the gene. Currently, the Inv22 can be diagnosed by a single-tube long-distance polymerase chain reaction (LD-PCR) when the causative mutation has not been established in an affected family, and it is not possible to analyze the entire FVIII gene intron 22, which is repeated twice extragenically (int22h-1 contains the polymorphic XbaI A and two constant sites that provide a control for XbaI digestion (restriction maps: GenBank entries AF062514, H86011 and H86012), Agarose gel electrophoresis (0.8%) of the PQ LD-PCR product digested with XbaI. Each pair of lanes contains undigested (nd) PCR product and corresponding digested (dig) product. Restriction patterns of homozygous [-/-], heterozygous [+/-] and homozygous [++] alleles are indicated in each case. Lane M corresponds to DNA size standard (λ HindIII). Due to ethidium-bromide staining, 4.8kb-long molecules will stain half as intensely as equal amounts of molecules of 9.6kb. In this method for X-genotyping, the 9.6kb-segment is cleaved into two fragments of 4.8kb, therefore the signals that represent the alleles [+], [-] from X [+/-] heterozygotes show equivalent fluorescent intensities. Amounts of molecules of 9.6kb. In this method for X-genotyping, the 9.6kb-segment is cleaved into two fragments of 4.8kb, therefore the signals that represent the alleles [+], [-] from X [+/-] heterozygotes show equivalent fluorescent intensities.

Specific M genotyping is achieved by nested PCR: aliquots (1 µL of 1:200 dilution) from the PQ LD-PCR product were subjected to nested PCR and analyzed as described Bowen et al.3 The 176bp DW-PCR product from int22h-1 contains the polymorphic MspI A and a constant site which provides a control for MspI digestion. Agarose gel electrophoresis (2%) of the DW PCR product digested with MspI. Lane M corresponds to a DNA size standard (100 bp ladder). Relevant signal sizes are indicated.

Donors: 2 males (X [-] and [+] ), 2 females ([+/-]), and 2 females ([+/-] and [+ +]) previously genotyped by validated methods. These last 2 individuals with genotype XbaI A [+] (and [+ +]) were also genotyped for the extragenic RFLPs XbaI BC [-] and [+ +]. The complete absence of [-] signal observed in their XbaI restriction analysis proves the specificity of the 12kb-amplimer for X, therefore for int22h-1 and consequently for M. Specific M genotyping is achieved by nested PCR: aliquots from the PQ LD-PCR product were subjected to a second round of PCR using primers DW,2 to generate an intragenic-specific 176bp-PCR product which presents all the requirements for reliable M genotyping (Figure 1B). The same series of six individuals used for X genotyping were employed to investigate M genotypes by nested PCR using the 12kb-product as a new specific substrate for the second round. Both methods, the validated one3 and the modification presented here, gave matching results.

Figure 1. Analysis of XbaI A (X) and MspI A (M) restriction fragment length polymorphisms (RFLPs). (a) X-RFLP analysis. Primers P and Q amplify 12kb from int22h-1 sequences. Optimal conditions for PQ LD-PCR were: 600 ng of genomic DNA, 50 mM Tris/ HCl pH 9.2, 1.75 mM MgCl2, 5% (v/v) dimethyl sulphoxide, 16 mM (NH4)2SO4, 0.6 µM of primers P and Q and 210 µM dGTP, 140 µM dATP, 150 µM dCTP and 2U TaqI Pwo DNA polymerase mixture (Expand long template DNA polymerases, Roche) in a volume of 20 µL. Three-temperature PCR were used for thermo-cycling. 3,6 PCR products (5-10 µL) were digested using SU of enzyme in 15 µL of total volume under the conditions recommended by the manufacturer (Promega or Gibco BRL). The figure shows a diagram of the PQ LD-PCR with XbaI restriction map of the product. The 12kb-product from int22h-1 contains the polymorphic XbaI A and two constant sites that provide a control for XbaI digestion (restriction maps: GenBank entries AF062514, H86011 and H86012). Agarose gel electrophoresis (0.8%) of the PQ LD-PCR product digested with XbaI. Each pair of lanes contains undigested (nd) PCR product and corresponding digested (dig) product. Restriction patterns of homozygous [-/-], heterozygous [+/-] and homozygous [++] alleles are indicated in each case. Lane M corresponds to DNA size standard (λ HindIII). Due to ethidium-bromide staining, 4.8kb-long molecules will stain half as intensely as equal amounts of molecules of 9.6kb. In this method for X-genotyping, the 9.6kb-segment is cleaved into two fragments of 4.8kb, therefore the signals that represent the alleles [+], [-] from X [+/-] heterozygotes show equivalent fluorescent intensities. 

Two sets of experiments were performed to adjust the PQ LD-PCR conditions: primer concentration (0.2, 0.4, 0.6, 0.8 and 1.0 µM) and thermo-cycling type (two, three-temperature and sub-cycling PCR).5 Optimal conditions for PQ LD-PCR are detailed in Figure 1A. The restriction patterns predicted for all X genotypes were verified using six DNA samples from healthy Argentinean blood donors: 2 males (X [-] and [+] ), 2 females ([+/-]), and 2 females ([+/-] and [+ +]) previously genotyped by validated methods. These last 2 individuals with genotype XbaI A [+] (and [+ +]) were also genotyped for the extragenic RFLPs XbaI BC [-] and [+ +]. The complete absence of [-] signal observed in their XbaI restriction analysis proves the specificity of the 12kb-amplimer for X, therefore for int22h-1 and consequently for M. Specific M genotyping is achieved by nested PCR: aliquots from the PQ LD-PCR product were subjected to a second round of PCR using primers DW,2 to generate an intragenic-specific 176bp-PCR product which presents all the requirements for reliable M genotyping (Figure 1B). The same series of six individuals used for X genotyping were employed to investigate M genotypes by nested PCR using the 12kb-product as a new specific substrate for the second round. Both methods, the validated one3 and the modification presented here, gave matching results.

Short amplifiers from heterogeneous-RFLP samples could undergo free association of their Watson and Crick strands to form the duplex. This phenomenon would produce equivalent amounts of each ([Watson:Crick], [:+:] and [:-:]) homoduplex and ([:+:] and [:+:]) heteroduplex. Among these four species only [:+:] homoduplex is cleaved. Although this effect does not interfere with M-genotyping, Figure 1B shows the 3:1 ([:+:]/[:+:]) signal-intensity ratio. Long-size amplifiers are not
Table 1. Frequencies and heterozygosities of XbaI/ MspI/ BclI haplotypes in the Argentinean population.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Marker/s</th>
<th>Heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>X M B</td>
<td>No %</td>
<td>O (%)</td>
<td>E (%)</td>
</tr>
<tr>
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<td>17 46</td>
<td>X 49</td>
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<td>0 0 M 50</td>
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<tr>
<td>– – –</td>
<td>13 35</td>
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</table>

Total 37 100

X, M and B code for markers XbaI, MspI and BclI (intron-18), respectively.

References