Polymerase chain reaction based human leucocyte antigen genotyping for the investigation of suspected gastrointestinal biopsy contamination

A C Bateman, S J Turner, J M Theaker, B F Warren, W M Howell

Abstract

Background—Mislabelling or contamination of surgical specimens may lead to diagnostic inaccuracy, particularly within gastrointestinal pathology when multiple small mucosal biopsy specimens are commonly taken, and where a tiny fragment of foreign tissue may be indistinguishable from true biopsy material using histological assessment alone.

Aims—To assess the utility of polymerase chain reaction (PCR) based human leucocyte antigen (HLA) genotyping techniques for the investigation of potentially mislabelled or contaminated gastrointestinal biopsy specimens.

Patients—Ten cases (28 samples) in which mislabelling or contamination was suspected, comprising four upper gastrointestinal tract biopsies and six colonoscopic biopsy series.

Methods—Direct and nested PCR-sequence specific primer (SSP) based HLA class II genotyping was performed on DNA extracted from formalin fixed and paraffin wax embedded tissue (23 samples) or peripheral blood leucocytes (five samples).

Results—A full HLA-DRB1 genotype was determined in all 28 samples. In seven cases the HLA-DRB1 genotype of the putative contaminant was different to that of the corresponding reference tissue, confirming different individual origins for the contaminant and reference material. In one case the contaminant tissue was shown to possess the same HLA-DRB1 alleles as a second patient (probable source). In the remaining three cases the same HLA-DRB1 alleles were detected within the potential contaminant and reference tissues.

Conclusions—PCR based HLA class II genotyping is a valuable tool for investigating potential contamination or mislabelling within gastrointestinal biopsy specimens and this report has confirmed contamination in seven of ten cases studied.

Keywords: polymerase chain reaction; human leucocyte antigen; tissue typing; contamination; mislabelling

Contamination or mislabelling of surgical pathology specimens can potentially lead to serious errors in diagnosis. Specimen contamination may pose a particular problem in gastrointestinal pathology because many specimens comprise multiple tiny mucosal biopsy samples and therefore a tiny tissue fragment that is “carried over” between specimens may closely resemble “true” biopsy fragments in size and nature. The individual origin of such contaminants is not likely to be ascertainable from histological assessment alone. Furthermore, many gastrointestinal biopsies are performed either when malignancy is clinically suspected or during screening for neoplasia, with such biopsies usually representing the gold standard for diagnosis. Therefore, specimen contamination or mislabelling could lead to assignment of a neoplastic condition to the incorrect patient, with potentially serious consequences for all involved.

We have assessed the utility of two previously described polymerase chain reaction (PCR) based human leucocyte antigen (HLA) class II genotyping methods for the investigation of ten potentially mislabelled or contaminated gastrointestinal biopsy cases. Both methods were developed within our laboratory (Histocompatibility and Immunogenetics, Southampton) and were initially derived from routine tissue typing techniques used for bone marrow and solid organ transplantation programmes and disease association studies. HLA class II genotyping was achieved in both techniques via a parallel series of PCRs, each of which contained a sequence specific primer (SSP) pair designed to produce a PCR product if a particular HLA class II allele or allele group was present (PCR-SSP genotyping). The nested PCR-SSP method incorporated an initial generic PCR amplification step in order to improve sensitivity significantly and to enable successful typing with very limited quantities of template DNA.

Case reports

CASE 1

A 66 year old woman underwent gastroscopy (distal oesophagitis but no obvious macroscopic lesion) and oesophageal biopsy during investigation for cervical lymph node and pulmonary metastatic carcinoma of unknown primary origin. Histology revealed one fragment of inflamed gastric type mucosa together with a fragment of severely dysplastic stratified
squamous epithelium, raising suspicion of contamination.

CASE 2
A 73 year old man underwent gastroscopy (no discrete macroscopic lesions) and two random biopsies for dyspepsia, one of which showed signet ring adenocarcinoma. Repeat gastroscopy confirmed no macroscopic evidence of tumour. Initial macroscopic and histological examination of the subsequent gastrectomy specimen revealed no evidence of tumour, raising the possibility that the initial biopsy was mislabelled or contaminated.

CASE 3
A 45 year old man underwent gastroscopy (duodenitis but no discrete lesions) for dyspepsia. Histology revealed gastritis together with a single tiny neoplastic epithelial fragment, raising suspicion of contamination.

CASE 4
A 70 year old woman with possible Crohn’s disease underwent colonoscopy and multiple biopsies for diarrhoea and right iliac fossa pain. One biopsy contained an additional dysplastic tissue fragment, raising suspicion of contamination.

CASE 5
A 20 year old woman with possible Crohn’s disease underwent colonoscopy and multiple biopsies for ulcerative colitis. The transverse colon biopsy specimen comprised dysplastic colonic epithelium. A colonic adenoma from a second patient was laboratory processed on the same day, raising suspicion that the dysplastic epithelium within the first patient’s biopsy had originated from the second patient.

DNA EXTRACTION—PERIPHERAL BLOOD SAMPLES
Where applicable (case 4), 1 ml of EDTA (Merck) blood from each individual underwent red cell lysis (buffer: 0.32 M sucrose (Sigma, Poole, UK), 1% Triton X-100 (Sigma), 5 mM MgCl₂ (Merck, Poole, UK), 12 mM Tris-HCl pH 7.5 (Sigma)) followed by 30 minute Proteinase K (Sigma) digestion, with a DNA yield of approximately 250 ng/µl.

DNA EXTRACTION—PARAFFIN WAX EMBEDDED TISSUE
As described previously, five 15 µm sections were cut from each paraffin wax block under investigation, and dewaxed. In one case (case 5) the putative contaminant was only present within one 5 µm haematoxylin and eosin stained paraffin wax section and was carefully scraped off the glass slide after cover slip removal. The resulting cellular material underwent overnight Proteinase K digestion. DNA concentration and integrity was estimated by running a 2 µl aliquot on a 2% agarose gel (Sigma).

NESTED PCR-SSP HLA CLASS II GENOTYPING
This method has been developed and previously described by our laboratory. The second exons within HLA-DRB1, 3, 4, and 5 genes were amplified in the first round PCR and 10 µl of reaction product was run on a 2% agarose gel to estimate DNA yield. This PCR product was used as the DNA template for the second round PCR-SSP step, generally at 1 in 10 000 dilution. Where the DNA yield from the initial PCR was low, dilutions of up to 1 in 20 were used, the optimum dilution being determined empirically. The PCR-SSP step was performed...
using panels of primers specific to hypervariable sequence motifs within the second exon of the HLA-DRB1 gene, plus primers specific for the HLA-DRB3, 4, and 5 genes. All primer sequences were derived from the literature, or were designed in house, using published HLA-DRB1 allele sequences, and were combined into 23 mixes, detecting 19 HLA-DRB1 alleles (or allele groups) plus the presence of the HLA-DRB3, -DRB4, or -DRB5 genes. PCR products were visualised by running the entire reaction mix on a 2% agarose gel. The PCR products were visualised by running the HLA-DRB3, -DRB4, or -DRB5 genes. All primer sequences were derived from the literature, or were designed in house, using published HLA-DRB1 allele sequences, and were combined into 23 mixes, detecting 19 HLA-DRB1 alleles (or allele groups) plus the presence of the HLA-DRB3, -DRB4, or -DRB5 genes. PCR products were visualised by running the entire reaction mix on a 2% agarose gel. The PCR products were visualised by running the entire reaction mix on a 2% agarose gel.

Table 1 PCR class II genotyping results

<table>
<thead>
<tr>
<th>Case</th>
<th>Method</th>
<th>HLA-DRB1</th>
<th>HLA-DRB3, 4, 5</th>
<th>HLA-DQB1</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neoplastic tissue nSSP 04, 13</td>
<td>53(4)†</td>
<td>NA</td>
<td>NA</td>
<td>Same HLA type</td>
</tr>
<tr>
<td>2</td>
<td>Reference tissue SSP 04, 13</td>
<td>53(4)</td>
<td>06, 08</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>3</td>
<td>Dysplastic tissue nSSP 0301, 15</td>
<td>51(5), 52(3)</td>
<td>NA</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>4</td>
<td>Reference tissue SSP 0301, 15</td>
<td>51(5), 52(3)</td>
<td>NA</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>5</td>
<td>Reference tissue SSP 0301, 14</td>
<td>52(3)</td>
<td>NA</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>6</td>
<td>Neoplastic tissue nSSP 0301, 04</td>
<td>52(3), 53(4)</td>
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<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>7</td>
<td>Reference (pb) SSP 04</td>
<td>53(4)</td>
<td>07, 08</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>8</td>
<td>Second patient nSSP 04</td>
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<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>9</td>
<td>Third patient nSSP 0301, 0701</td>
<td>53(4)</td>
<td>NA</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>10</td>
<td>Fourth patient (pb) SSP 0301, 13</td>
<td>52(3)</td>
<td>02, 06</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>11</td>
<td>Fifth patient (pb) SSP 0301, 13</td>
<td>52(3)</td>
<td>02, 06</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>12</td>
<td>Sixth patient (pb) SSP 0301, 1302</td>
<td>52(3)</td>
<td>02, 06</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>13</td>
<td>Seventh patient (pb) SSP 0301, 1302</td>
<td>52(3)</td>
<td>02, 06</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>14</td>
<td>Dysplastic tissue nSSP 0103, 0301</td>
<td>52(3)</td>
<td>NA</td>
<td></td>
<td>Same HLA type</td>
</tr>
<tr>
<td>15</td>
<td>Reference tissue SSP 01, 1402</td>
<td>52(3)</td>
<td>05, 07</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>16</td>
<td>Dysplastic tissue nSSP 01, 04</td>
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</tr>
<tr>
<td>17</td>
<td>Reference tissue SSP 012, 1302</td>
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<td>06, 07</td>
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</tr>
<tr>
<td>18</td>
<td>Dysplastic tissue nSSP 0301, 13</td>
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<td>NA</td>
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<td>Contaminant</td>
</tr>
<tr>
<td>19</td>
<td>Reference tissue SSP 0301, 14</td>
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<td>02, 06</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>20</td>
<td>Dysplastic tissue nSSP 01, 15</td>
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<td>NA</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>21</td>
<td>Reference tissue SSP 15, 15</td>
<td>51(5)</td>
<td>06</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>22</td>
<td>Second patient SSP 01, 15</td>
<td>51(5)</td>
<td>07, 08</td>
<td></td>
<td>Contaminant</td>
</tr>
<tr>
<td>23</td>
<td>Remaining tissue nSSP 03, 14</td>
<td>52(3)</td>
<td>02</td>
<td></td>
<td>Contaminant</td>
</tr>
</tbody>
</table>

SSP, direct PCR-SSP genotyping; nSSP, nested PCR-SSP genotyping; pb, peripheral blood derived DNA (all other results derived from formalin fixed, paraffin wax embedded DNA).
†The expected DQB1*05 allele was not detected.
‡The expected DRB3 (DR52) gene was not detected.

In cases 1, 2, and 5, the chance of two unselected white Western individuals possessing the same HLA-DRB1 alleles is: case 1 (HLA-DRB1*04, 13) 6%; case 2 (HLA-DRB1*0301, 15) 8%; case 5 (HLA-DRB1*0301, 0301) <1%.1, 2

Discussion

Specimen mislabelling or contamination can occur at any stage from the clinic or operating theatre to within the histopathology laboratory and may lead to serious diagnostic uncertainty or error. We have previously developed PCR based HLA class II tissue genotyping methods that were derived from routine DNA based tissue typing techniques for solid organ and bone marrow transplantation matching and disease association studies1, 2 and are applicable to the relatively degraded DNA extractable from formalin fixed and paraffin wax embedded tissue.
We have also developed related PCR-based techniques, again applicable to paraffin wax biopsy specimens, for tissue identification and HLA-disease association studies. These techniques are particularly useful for the investigation of individual tissue identity as HLA molecules, involved in modulation of the immune response, are encoded by the most polymorphic loci in the human genome, within the major histocompatibility complex on chromosome six. For example, there are currently 184 identified DRB1 alleles and 31 DQB1 alleles. HLA encoding genes do not seem to undergo somatic mutation during neoplastic transformation. In fact, only one instance of somatic mutation of an HLA allele has been described, in a case of renal cell carcinoma. Therefore, the fact that many of the tissue fragments undergoing HLA genotyping in this study were neoplastic in nature is not likely to have detracted from the accuracy of the method for determining individual identity.

Identification of different HLA alleles for the locus studied within putative contaminant and reference tissues would therefore indicate different individual origins for the tissues and confirm that contamination or mislabelling had occurred. Conversely, identification of the same HLA alleles within two tissue fragments suggests that they are most likely to be derived from the same individual. However, in this situation it is possible that the two fragments may be derived from different individuals possessing the same detected HLA alleles for the loci examined. The chance of this occurrence depends on the frequency of the HLA alleles in the general population. The HLA-DRB1 alleles vary in frequency among Western Caucasians from 0.7% for HLA-DRB1*10 to 33% for HLA-DRB1*04 and 36% for HLA-DRB1*15, but as an individual possesses two alleles for each HLA locus, the frequency of possession of a combination of two alleles will be significantly less than the frequency of the individual alleles. For example, the frequency of the HLA-DRB1*15, 15 homozygote genotype is approximately 13%. In this study, the frequency of the HLA-DRB1*15, 15 homozygote genotype is approximately 13%. In the remaining seven cases, contamination was confirmed by the detected presence of at least one different HLA-DRB1 allele between the contaminant and reference tissues, enabling erroneous suspicion of dysplasia or malignancy to be avoided in these patients. These patients could therefore be reassured to a degree, although clinical follow up was continued. In case 1, the metastatic carcinoma within the cervical lymph node was subsequently confirmed as adenocarcinoma and together with the endoscopic appearance of the oesophagus, the fragment of dysplastic squamous epithelium was still believed clinically most likely to represent a contaminant. In this case HLA class II genotyping led to confirmation of the diagnosis of malignancy in this patient and therefore helped to exclude the important possibility that the initial malignant gastric biopsy specimen was derived from a second patient. Although HLA class II genotyping could not prove contamination in case 5, the presence of the dysplastic mucosal fragment on only a single histological section was strongly suggestive of "carry over". Therefore, this patient could be reassured to a degree, although clinical follow up was continued. In this case HLA class II genotyping led to confirmation of the diagnosis of malignancy in this patient and therefore helped to exclude the important possibility that the initial malignant gastric biopsy specimen was derived from a second patient. Although HLA class II genotyping could not prove contamination in case 5, the presence of the dysplastic mucosal fragment on only a single histological section was strongly suggestive of "carry over". Therefore, this patient could be reassured to a degree, although clinical follow up was continued. In this case HLA class II genotyping led to confirmation of the diagnosis of malignancy in this patient and therefore helped to exclude the important possibility that the initial malignant gastric biopsy specimen was derived from a second patient. Although HLA class II genotyping could not prove contamination in case 5, the presence of the dysplastic mucosal fragment on only a single histological section was strongly suggestive of "carry over". Therefore, this patient could be reassured to a degree, although clinical follow up was continued. In this case HLA class II genotyping led to confirmation of the diagnosis of malignancy in this patient and therefore helped to exclude the important possibility that the initial malignant gastric biopsy specimen was derived from a second patient. Although HLA class II genotyping could not prove contamination in case 5, the presence of the dysplastic mucosal fragment on only a single histological section was strongly suggestive of "carry over". Therefore, this patient could be reassured to a degree, although clinical follow up was continued. In this case HLA class II genotyping led to confirmation of the diagnosis of malignancy in this patient and therefore helped to exclude the important possibility that the initial malignant gastric biopsy specimen was derived from a second patient.

In summary, this study illustrates the particular utility of PCR-SSP HLA class II genotyping for the investigation of potentially mislabelled or contaminated gastrointestinal biopsy samples, specimens in which a diagnosis of neoplasia is often made on minimal tissue and with major implications for the patients involved. The nested modification of the method is particularly applicable to the small
quantities of DNA extractable from tiny formalin fixed and paraffin wax embedded endoscopic biopsy specimens.

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