**ELECTRONIC LETTER**

Mutational analysis of N-ras, p53, CDKN2A (p16<sup>INK4a</sup>), p14<sup>ARF</sup>, CDK4, and MC1R genes in human dysplastic melanocytic naevi

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In order to detect possible dysplastic melanocytic naevi (DMN) associated melanoma risk factors and lesion specific differences in the mutation spectrum of dysplastic and congenital melanocytic naevi (CMN), we screened 19 specimens of human sporadic DMN derived from 19 patients for the presence of mutations in five genes, which we had investigated in a former study in 19 CMN and which have been reported to be associated with human cutaneous melanoma (N-ras, p53, CDKN2A, CDK4, and MC1R).

The screening strategy for the detection of activating point mutations in the oncogenes N-ras and CDK4 as well as for germline sequence variants in the MC1R gene by combined RFLP-PCR/SSCP analysis, and the screening strategy for the detection of homozygous deletions and point mutations in the tumour suppressor genes p53 and CDKN2A by combined multiplex-PCR/SSCP analysis, have been described previously. In order to find out if the SSCP screening system that is used in our laboratory is suitable to detect point mutations in minor cellular subpopulations of the DMN lesions investigated (for example, N-ras point mutations in the atypical melanocytic fraction), we added gradually decreasing amounts of N-ras mutation harbouring genomic DNA (CAA to AAA mutation at one allele) to genomic N-ras wild type DNA before PCR and SSCP analysis. As a result, we could show that the aberrant mutation associated SSCP band is still visible at an admixture of less than 1% of mutation harbouring DNA.

In the present study we extended our MC1R screening system in order to allow the detection of two additional sequence variants (R151C and R160W), which like V92M and D294H have been reported to be associated with red hair and light skin.

Table 1 N-ras mutations and MC1R variants in human spontaneous dysplastic naevi

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Gender</th>
<th>Age</th>
<th>Lesion (mm)</th>
<th>Location/ subtype</th>
<th>% of AM</th>
<th>Skin type</th>
<th>Naevus colour</th>
<th>N-ras mutation</th>
<th>MC1R variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>33</td>
<td>6</td>
<td>Back CN</td>
<td>10</td>
<td>NA</td>
<td>Light brown</td>
<td>No</td>
<td>R151C</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>49</td>
<td>NA</td>
<td>Upper leg CN</td>
<td>10</td>
<td>II</td>
<td>Brown</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>39</td>
<td>9</td>
<td>Back CN</td>
<td>20</td>
<td>NA</td>
<td>Dark brown</td>
<td>No</td>
<td>No</td>
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<tr>
<td>4</td>
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<td>13</td>
<td>ND</td>
<td>Back CN</td>
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<tr>
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<td>37</td>
<td>NA</td>
<td>Upper arm CN</td>
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<td>Brown</td>
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</tr>
<tr>
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<td>Male</td>
<td>29</td>
<td>5</td>
<td>Back CN</td>
<td>30</td>
<td>II</td>
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<td>No</td>
<td>R151C</td>
</tr>
<tr>
<td>7</td>
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<td>55</td>
<td>3</td>
<td>Ear JN</td>
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<tr>
<td>8</td>
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<tr>
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<td>26</td>
<td>NA</td>
<td>Back CN</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
<td>D84E</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>23</td>
<td>NA</td>
<td>NA CN</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
<td>No</td>
</tr>
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<td>35</td>
<td>4</td>
<td>Back CN</td>
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<td>Brown</td>
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</tr>
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<td>38</td>
<td>NA</td>
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<td>No</td>
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<td>4</td>
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<td>Dark brown</td>
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<td>No</td>
</tr>
<tr>
<td>16</td>
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<td>23</td>
<td>3</td>
<td>Upper leg JN</td>
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<td>No</td>
<td>V92M</td>
</tr>
<tr>
<td>17</td>
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<td>47</td>
<td>NA</td>
<td>Flank CN</td>
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<td>NA</td>
<td>Brown</td>
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<td>No</td>
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<tr>
<td>18</td>
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<td>33</td>
<td>NA</td>
<td>Back JN</td>
<td>10</td>
<td>NA</td>
<td>CAA (Q) to AAA (K)</td>
<td>No</td>
<td></td>
</tr>
<tr>
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<td>Sole CN</td>
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<td>NA</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

NA = information not available, % of AM = % of histologically characterised atypical melanocytes in the respective DMN lesion; subtype = histological subtype; CN = compound naevus, JN = junction naevus.
cord 160 mutation screening), 10 µl of preamplification solution 'was used as a template under otherwise identical PCR conditions. CfoI (GGC/C) and KspI (CCG/C) digestion for restriction enzyme based mutation screening of codon 151 (resulting in 40, 31, and 49 bp fragments) and codon 160 (resulting in 66 and 54 bp fragments), respectively, was performed according to the recommendations of the supplier (Roche). In the present study, we also screened all DMN lesions for homozygous deletion and mutations in exon 1 of the p14ARF tumour suppressor gene. The p14ARF protein is induced by oncogenic stimuli and then prevents MDM2 mediated p53 ubiquination/degradation. For exon 1β preamplification, we used the p16E2 (509 bp)/p53E8 (245 bp) multiplex PCR assay as described in Papp et al. in combination with a third primer pair, p14ARF2A (5′ CTAC TCTCTG GTGCC AAAGG 3′)/p14ARF2B (5′ CCTAG AATGG GCTAG AGACG 3′), which results in the generation of an additional 348 bp fragment (triplex PCR). For reamplification and subsequent SSCP analysis, 10 µl of the preamplification solution was used as template for a 280 bp semi-nested PCR product using primer pair p14ARF2A (5′ CTAC TCTCTG GTGCC AAAGG 3′)/p14ARF T1 (5′ GACTT TTCGA GGGCC TTTCC 3′) and 25 cycles under otherwise identical PCR conditions. Ten µl of the reamplification product were digested with KspI (CCGC/GG, 149 and 131 bp fragments) according to the recommendations of the supplier (Roche) before SSCP analysis.

RESULTS

In our present investigation no mutations could be detected in CDKN2A, in exon 1β of the p14ARF gene, in p53, or in CDK4. These results are consistent with the low frequency of reported mutations in these genes in different histotypes of sporadic primary human cutaneous melanomas (SSM, NNM, and LMM) and melanoma metastases. Only one activating point mutation in the N-ras gene (a CAA (Q) to AAA (K) transversion in codon 61) in one of 19 lesions (5%) could be found. In the MC1R gene four missense mutations in a heterozygous state could be found in four lesions (two R151C, one V92M, and one D84E mutation). The D84E variant has been reported to be associated with melanoma. In patient 6, the R151C polymorphism is associated with skin type II (table 1).

DISCUSSION

An interesting point arises when comparing the N-ras mutation frequencies in DMN and CMN. A very low frequency of N-ras mutations seems to be characteristic of DMN, whereas quite the opposite seems to be the case for CMN. In the present study, we found only one N-ras mutation in 19 screened DMN lesions (table 1). Furthermore, in former studies 49 DMN were investigated by three independent groups for point mutations in the N-ras gene in codons 12, 13, and 61 and no point mutations were detected. On the other hand, in a more recent CMN study, we found N-ras mutations with a so far unprecedented high frequency (56%). Consistent with our findings, Carr et al. detected 12 activating N-ras mutations in 43 investigated CMN (28%) by a dot blotting/oligo probing technique (six CGA, five AAA, and one CTA mutation).

Because for nodular malignant melanoma (NMM) an association with N-ras mutations was reported by different authors, it may be conceivable that an aetiologically based connection could exist between CMN and NMM rather than between DMN and NMM.

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Figure 1 Silver stained SSCP gel comprising PCR products using N-ras wild type DNA with decreasing admixture of CAA to AAA N-ras mutated DNA (100%-0.8% admixture) as a template together with size marker and pure N-ras wild type DNA as a negative control (0%). The mutation indicating band is still visible using an admixture of less than 1% mutation carrying DNA (see arrow).
REFERENCES


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