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

T Papp, H Pemsel, I Rollwitz, H Schipper, D G Weiss, D Schiffmann, R Zimmermann

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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<sup>1</sup>, p53<sup>2</sup>, CDKN2A<sup>3</sup>, CDK4<sup>4</sup>, and MC1R<sup>5</sup>).

METHODS

DNA was extracted from selected paraffin embedded DMN resection specimens using the QIAamp DNA Mini Kit (Qiagen) according to the recommendations of the supplier. The relative number of atypical melanocytes in the DMN and the histological subtype of the DMN were determined in parallel slides by an experienced dermatologist (Dr Regina Zimmermann) (table 1).

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.<sup>6</sup> 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 (fig 1).

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.<sup>7</sup> For reamplification of the 899 bp MC1R preamplification product with primer pair MC1R4A (5′ TCGGC GTGGA CCGGT ACATC 3′)/MC1R4B (5′ GCCG CGTGA GACGA CACTG 3′) (120 bp PCR product, suitable for codon 151 and

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<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>
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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.
codon 160 mutation screening), 10 µl of preamplification solution was used as a template under otherwise identical PCR conditions. CfoI (CGG/C) and KspI (CCGGGG) 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 T.1 protein, 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 a template under otherwise identical PCR conditions. Ten µl of the preamplification solution was used for restriction enzyme based mutation screening of codon 151 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.11–13

On the other hand, in a more recent CMN study,14 we found N-ras mutations with a so far unprecedented high frequency (56%). Consistent with our findings, Carr et al.12 detected 12 activating N-ras mutations in 43 investigated CMN (28%) by a dot blotting/oligo probing technique (six CAA, 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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