ELECTRONIC LETTER

Mutational analysis of *N-ras*, *p53*, *CDKN2A* (*p16^{INK4a}*), *p14^{ARF}*, *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[1] and which have been reported to be associated with human cutaneous melanoma (*N-ras*,[2] *p53*,[3] *CDKN2A*,[4] *CDK4*,[5] and *MC1R*[6 7]).

Key points

- Nineteen specimens of human sporadic dysplastic melanocytic naevi (DMN) were screened for the presence of mutations in *N-ras*, *p53*, *p16INK4a*, *p14ARF*, *CDK4*, and *MC1R*.
- In contrast to human congenital melanocytic naevi, a very low frequency of *N-ras* mutations seems to be characteristic of DMN.

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.[1] 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 melanocyte fraction), we added gradually decreasing amounts of *N-ras* mutation harbouring genomic DNA (CAA to **A**AA 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[6] have been reported to be associated with red hair and light skin.[8] For reamplification of the 899 bp *MC1R* PCR preamplification product[1] with primer pair MC1R4A (5′ TCGCC GTGGA CCGCT ACATC 3′)/MC1R4B (5′ GCGTG CTGAA GACGA CACTG 3′) (120 bp PCR product, suitable for codon 151 and

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

Patient No	Gender	Age	Lesion (mm)	Location/ subtype	% of AM	Skin type	Naevus colour	*N-ras* mutation	*MC1R* variant
1	Female	33	6	Back CN	10	NA	Light brown	No	R151C
2	Female	49	NA	Upper leg CN	10	II	Brown	No	No
3	Female	39	9	Back CN	20	NA	Dark brown	No	No
4	Male	13	ND	Back CN	5	NA	Brown	No	No
5	Female	37	NA	Upper arm CN	5	NA	Brown	No	No
6	Male	29	5	Back CN	30	II	Dark brown	No	R151C
7	Male	55	3	Ear JN	40	NA	Dark brown	No	No
8	Male	19	NA	Breast CN	1	NA	NA	No	No
9	Female	26	NA	Back CN	5	NA	NA	No	D84E
10	Female	23	NA	NA CN	10	NA	NA	No	No
11	Male	35	4	Back CN	5	NA	Brown	No	No
12	Male	35	NA	Back CN	5	NA	Brown	No	No
13	Female	38	NA	Back CN	10	NA	NA	No	No
14	Female	27	5	Upper leg JN	20	NA	Brown	No	No
15	Male	54	4	Back CN	10	NA	Dark brown	No	No
16	Female	23	3	Upper leg JN	5	NA	Dark brown	No	V92M
17	Female	47	NA	Flank CN	5	NA	Brown	No	No
18	Female	33	NA	Back JN	10	NA	NA	CAA (Q) to **A**AA (**K**)	No
19	Female	19	NA	Sole CN	5	NA	NA	No	No

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.

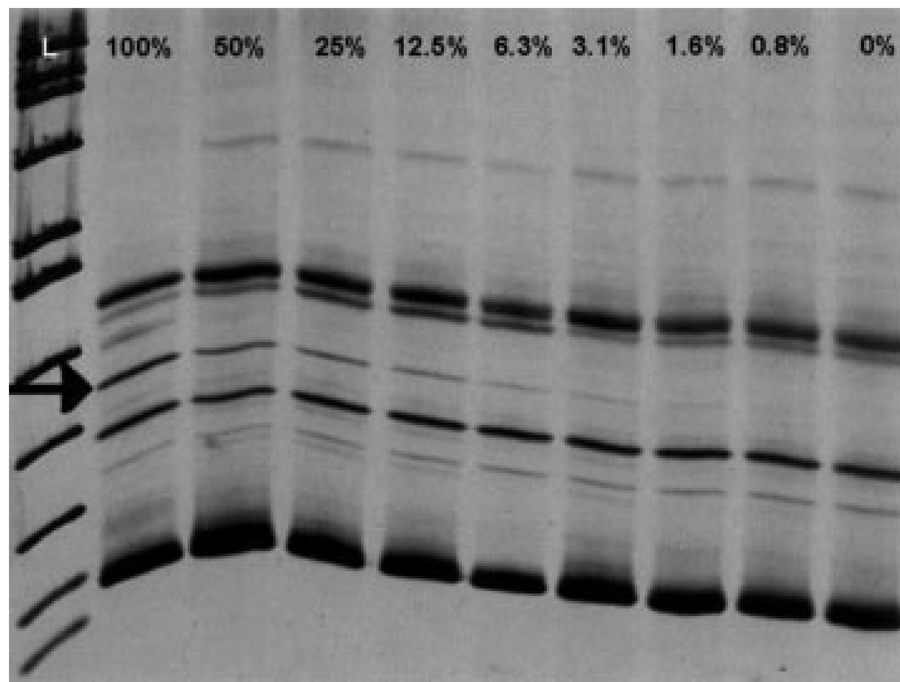

Figure 1 Silver stained SSCP gel comprising PCR products using *N-ras* wild type DNA with decreasing admixture of CAA to AAA *N-ras* mutated DNA (100%-0.8% admixture) as a template together with size marker and pure *N-ras* wild type DNA as a negative control (0%). The mutation indicating band is still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

codon 160 mutation screening), 10 µl of preamplification solution[1] was used as a template under otherwise identical PCR conditions. *Cfo*I (G**CG**!**C**) and *Ksp*I (CCG**C**!**GG**) 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 1β of the *p14*[ARF] tumour suppressor gene. The p14[ARF] protein is induced by oncogenic stimuli and then prevents MDM2 mediated p53 ubiquination/degradation.[9] For exon 1β preamplification, we used the p16E2 (509 bp)/p53E8 (245 bp) multiplex PCR assay as described in Papp *et al*[10] in combination with a third primer pair, p14ARF2A (5′ CTCAC CTCTG 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′ CTCAC CTCTG GTGCC AAAGG 3′)/ p14ARF T.1 (5′ GACTT TTCGA GGGCC TTTCC 3′) and 25 cycles under otherwise identical PCR conditions. Ten µl of the reamplification product were digested with *Ksp*I (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 *p14*[ARF] 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.[3–5]

Only one activating point mutation in the *N-ras* gene (a CAA (Q) to **A**AA (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.[7] 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.[11–13]

On the other hand, in a more recent CMN study,[1] 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 C**G**A, five **A**AA, and one C**T**A mutation).

Because for nodular malignant melanoma (NMM) an association with *N-ras* mutations was reported by different authors,[13 14] 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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