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Melanoma and nonmelanoma skin cancer in patients with multiple tumours—evidence for new syndromes in a population-based study

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Summary

Background The hypotheses that Swedish patients with four or more primary tumours [including at least one cutaneous malignant melanoma (CMM)] harbour an increased number of *CDKN2A* (formerly *p16*) germline mutations, and that this group of patients show a predisposition to other tumours, e.g. nonmelanoma skin cancer (NMSC), were studied descriptively. So far the mutation *113insArg* explains all *CDKN2A*-associated CMM in ethnic Swedes.

Objectives All patients with four or more primary tumours, of which at least one was a CMM, from the Southern Swedish Regional Tumour Registry, between 1958 and 1999, were included in this study.

Methods Forty-four patients were found and subdivided into three groups according to having multiple CMM (group A) or single CMM ± NMSC (groups B and C). Screening for the presence of the Swedish founder mutation *113insArg* in blood or in tissue blocks was performed.

Results Patients in group A were younger at the time of the first CMM diagnosis than patients in group B and group C. The *113insArg* mutation was found in four of 44 patients (9%), three with multiple CMM. In group C ($n = 14$) no founder mutation was evident, while in group B ($n = 15$) one mutation carrier was found. Nonmutation carriers with multiple CMM (group A) also had a predilection for meningiomas and neurinomas (four patients) or multiple NMSC (three patients). In group B CMM were especially associated with adenocarcinomas but in group C CMM were associated with multiple NMSC.

Conclusion The association between meningiomas and neurinomas (no acoustic neurinoma was seen) might indicate a new syndrome. Patients in groups B and C may harbour unknown genetic defects, which could interact with different environmental risk factors.

Key words: *CDKN2A*, CMM and associated tumours, multiple melanoma, mutation analysis, NMSC, *p16*

Patients with multiple primary tumours are an important group for epidemiological studies and it is conceivable that multiple primary tumours in one patient at a young age may have one or several underlying genetic causes.[1–4] According to the literature *CDKN2A* (formerly *p16*) germline mutations can be over-represented in patients with multiple primary cutaneous malignant melanomas (CMM).[5–8] Our first hypothesis was that patients with four or more primary tumours, of which at least one was a CMM, and especially patients with multiple CMM, might include a high proportion of

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mutation carriers. Screening for *113insArg* was desirable in this study as this mutation in the *CDKN2A* suppressor gene (formerly *p16*) so far explains all *CDKN2A*-associated CMM in ethnic Swedes. As ultraviolet radiation (UV) exposure is a joint risk factor for both CMM and NMSC,[9] our second hypothesis was that patients who developed both CMM and NMSC would be older than hereditary *CDKN2A* cases when CMM was first diagnosed and would show associated tumours that could reveal important new environmental and/or genetic factors. The third hypothesis was that CMM and adenocarcinomas might be associated, based on the findings of an association with CMM and, for example, primary breast, or colon cancer as previously described.[10–16] We also wanted to evaluate whether patients with at least four primary tumours including multiple CMM showed different tumour aggregations or associations and different mutation status than patients with four or more primary tumours including a single CMM.

Materials and methods

Patients

The South Swedish Health Care Region comprises approximately 1·5 million inhabitants. All patients in the Southern Swedish Regional Tumour Registry diagnosed between 1958 and 1999 with four or more primary tumours ($n = 394$) of the same or different origin and of which one or more was a malignant melanoma were included in the study. Forty-four patients met the criteria, including 26 males and 18 females. The date of birth ranged from 1877 to 1948. At the time the data were collected 13 patients were alive and 31 patients had died. Invasive tumours as well as *in situ* lesions (involving all diagnoses) were included. All NMSC were squamous cell carcinomas. The patients were subdivided into three groups (based on the hypotheses mentioned above) that included multiple CMM ± NMSC and ± other tumours (group A), single CMM and other tumours (group B) or single CMM and NMSC ± other tumours (group C). The groups were further studied descriptively.

Cancer Registry

The Swedish Cancer Registry at the National Board of Health and Welfare was started in 1958 and since then all new cases of cancer in Sweden have been reported and recorded. A unique 10-digit national registration number ascribed to every citizen ensures accurate identification in the registration of all diagnosed tumours.

Reporting to the regional Registry by both the clinician who diagnoses a malignant disease and by the pathologist, who must report separately any diagnosis of cancer made on pathological and cytological specimens, is compulsory. An almost 100% coverage in cancer registration[6,17,18] has been achieved with this compulsory reporting by two clinicians.

The Swedish Cancer Registry also includes all tumours of the central nervous system including intracranial and intraspinal tumours, neurinomas and all endocrine tumours, e.g. pituitary adenoma. Basal cell carcinomas are not reported. Hence a few diagnoses reported are not malignant but benign tumours, e.g. meningiomas and pituitary adenomas. Multiple primary tumours are registered in accordance with the internationally proposed rules.[18–20]

Mutation screening

Paraffin-embedded tissue blocks were obtained from 41 of the 44 individuals and peripheral blood samples were obtained from one patient for DNA extraction and mutation analysis. This restricted testing to that for the known Swedish founder mutation *113insArg* only and not for the whole genome. For two patients neither tumour block nor blood could be retrieved and so they were not tested.

Polymerase chain reaction with a mutation-specific primer

The polymerase chain reaction analysis was performed as described by Borg *et al.*[11]

Statistical analysis

The difference in age distribution and mutation prevalence between the patient groups was analysed with the Wilcoxon test and exact odds ratios (OR) and confidence intervals (CI) were calculated between the groups. Age- and sex-specific expected values were calculated for NMSC and for tumours of the nervous system corresponding to the International Classification of Diseases, Seventh revision, codes 191 and 193, respectively.

Results

Three of the 44 patients were recognized as belonging to the South Swedish Melanoma Families with a

previously known family history of malignant melanoma and the known founder mutation *113insArg*, characterized by Borg *et al.*[11,21] (Fig. 1).

Comparison between groups

Associated tumour types differed between the groups and are presented as the number of patients with diagnoses and median ages in Figure 2.

In group A with multiple CMM, meningiomas ($n = 2$, two patients with a primary tumour), neurinomas ($n = 2$, two patients with a primary tumour) and NMSC ($n = 6$, six patients with 20 primary tumours, of whom three patients had multiple NMSC), were found. There were three mutation carriers but none was among the patients with a neurinoma, a meningioma or with multiple NMSC.

In group B patients with adenocarcinomas (prostate $n = 5$, five patients each with a primary tumour; breast $n = 5$, five patients with nine primary tumours; kidney $n = 4$, four patients with six primary tumours; colon $n = 3$, three patients with five primary tumours; gynaecological tumours $n = 3$, three patients with a primary tumour each; pancreas $n = 2$, two patients each with a primary tumour and small bowel carcinoid $n = 2$) occurred in combination with single melanomas. One patient had the *113insArg* mutation. No patient had NMSC. Because the expected rate can only be calculated for site but not for histological type, the expected rate for development of adenocarcinomas in a normal population could not be calculated. It was not possible to elucidate the cause of death for each patient.

In group C, patients with a single melanoma also developed one or multiple NMSC (14 patients with 31 primary NMSC). Three patients also developed rectal carcinoma (three patients each with a primary tumour). No patient had the *113insArg* founder mutation. Lympho/haematopoietic tumours as well as prostate tumours presented in all groups at a low frequency.

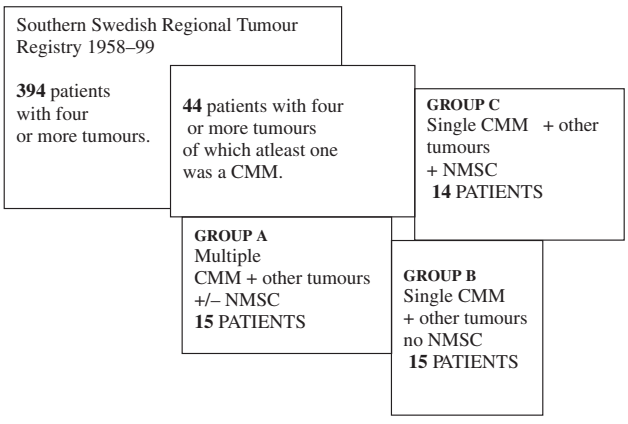

Figure 1. Of 394 patients with multiple tumours, 44 patients had at least one cutaneous malignant melanoma (CMM). Fifteen patients had multiple CMM; another 15 patients had a single CMM, several other tumours but no nonmelanoma skin cancer (NMSC). The last 14 patients had a single CMM, one or several NMSC as well as other nonskin cancer diagnoses.

Expected rate for the development of tumours

Significantly more neural tumours and NMSC, respectively, were seen in group A; five observed cases vs. 0·1 expected ($P < 0{\cdot}0001$) and six observed vs. 0·2 expected ($P < 0{\cdot}0001$). In group C significantly more NMSC were seen (14 observed cases vs. 0·5 expected, $P < 0{\cdot}0001$) (Fig. 2).

Age

Patients in group A developed their first CMM at a younger median age (60 years), than patients in group C (77·5 years, $P < 0{\cdot}005$). No statistically significant difference in age at first CMM presentation was seen between groups A and B ($P = 0{\cdot}21$). A significant difference was also seen between group B and group C; (67 years vs. 78 years, $P < 0{\cdot}04$) (Fig. 2).

The median age (with 95% CI) for developing CMM, NMSC, other tumours and death, respectively, are shown in Figure 2 for each group.

Identification of the CDKN2A mutation

The Swedish founder mutation *113insArg* was detected in four (9%) of the 44 patients. Three were in group A and one in group B. No case was found in group C. Two of the three mutation carriers in group A as well as the patient in group B were found to belong to South Swedish Melanoma families already reported.[11,21]

The OR of carrying the founder mutation was calculated: (i) A vs. C; OR = 8·1 (CI 0·5–∞); (ii) A vs. B; OR = 3·5 (CI 0·3–96·6); (iii) B vs. C; OR = 2·0 (CI 0·02–41·2).

Discussion

We hypothesized, according to previous publications on multiple CMM, that the occurrence of four or more primary tumours in the same individual might to a

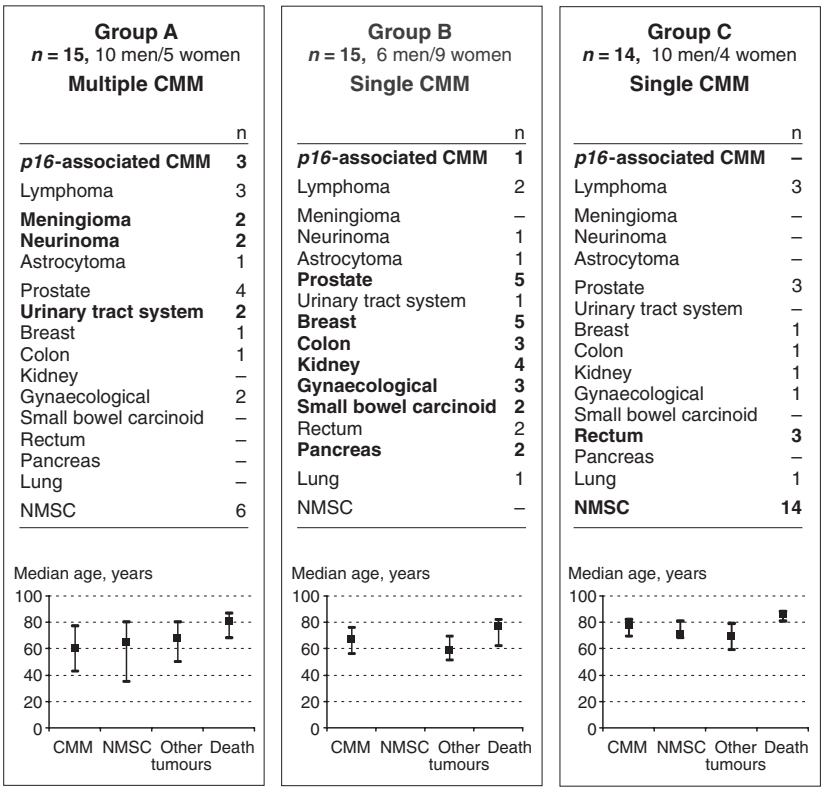

Figure 2. Numbers of patients, sex ratio, number of *p16* mutation carriers, associated tumours and median age with confidence intervals for groups A, B and C, are presented. Total number of diagnoses (if multiple primaries) are not presented here. The expected rate for neural tumours in a normal population comparable with group A: 0·1 ($P < 0{\cdot}0001$). The expected rate for nonmelanoma skin cancers (NMSC) in a normal population comparable with group A: 0·2 ($P < 0{\cdot}0001$). The expected rate for neural tumours in a normal population comparable with group B: 0·2 ($P = 0{\cdot}04$). The expected rate for NMSC in a normal population comparable with group B: 0·2 ($P = 0{\cdot}8$). The expected rate for neural tumours in a normal population comparable with group C: 0·2 ($P = 0{\cdot}8$). The expected rate for NMSC in a normal population comparable with group C: 0·5 ($P < 0{\cdot}0001$). CMM, cutaneous malignant melanoma; NMSC, nonmelanoma skin cancer; *p16*, *CDKN2A* gene.

certain extent result from a genetic predisposition to tumour development. We assumed that this tumour susceptibility might, in some cases and especially when there were multiple CMM, be due to germline *CDKN2A/p16* mutations.[2,5–7,20,22–27] In Sweden the founder mutation *113insArg* is so far the only known important mutation relating to melanomas. As the material was based mainly on tissue blocks, the screening could not be extended to test the whole genome but only the known founder mutation.

Our second and third hypotheses were based on the idea that patients with single CMM and three or more other primary tumours might be part of unknown or poorly known syndromes and/or reveal new possible environmental or genetic tumour-predisposing factors. There might for instance be a relationship between late melanomas, DNA repair defects and multiple NMSC.

In fact we found that the mutation mainly occurred in patients with multiple melanomas (group A). We also found a possible relationship between multiple melanomas and neurinomas/meningiomas in nonmutation carriers. None of the neurinomas was an acoustic neurinoma and it is not likely that these individuals carry mutations in the gene for the neurofibromatosis 2 syndrome, but our findings might instead indicate a possible new syndrome. A conceivable reason for this association might be the common embryological origin of melanocytes and nervous system cells, all derived from the neural crest. Earlier a few other authors have seen a relationship between CMM, neural tumours and *CDKN2A* germline deletions.[28,29]

Adenocarcinomas were most common among patients in group B. In group C the associated tumours were mostly multiple NMSCs but a slightly higher occurrence (compared with the other groups) of rectal tumours was also observed. Lymphomas occurred equally distributed in all three groups among nonmutation carriers.

This study is a descriptive, registry-based investigation. A general limitation of this type of study is the lack of information about the exact exposure to hypothesized aetiological factors, e.g. smoking status, cancer treatment, etc., at the individual level. One should keep this in mind to avoid over-interpretation of the associations observed.[20] We should also emphasize that although we are using the entire dataset from a population-based Cancer Registry, the number of patients in each category is small with the risk of over-interpretion of the data; our findings need to be confirmed with larger datasets.

Multiple NMSC were most common in group C and in a small subgroup of patients in group A, supporting the distinction of the groups and possible difference in aetiology. In addition, a significant age difference in first CMM diagnosis was evident between groups A and C as well as between groups B and C.

The study of multiple primary cancers of a specific tumour type could give great insights into the importance not only of particular genetic markers, but also of their interactions with other genetic characteristics (e.g. *CDKN2A/p16* mutations) and of their interactions with environmental exposures (e.g. *CDKN2A/p16* mutations and various levels of sun exposure).[30] The identification of patients with different genetic lesions that pose a substantial risk of a second primary cancer could have considerable clinical value, prompting appropriate surveillance, not just for the affected patient but also for other family members.[25] As survival after several malignancies increases, the incidence of multiple primary tumours will also increase, especially as some multiple primary tumours may be caused by late therapeutic side-effects.[31,32]

The causes of CMM and NMSC are not totally understood and are aetiologically multifactorial. The interactions between UV exposure and genetic predisposition may be the critical factor. It has become clear that the genes involved in the genetic basis of melanoma may play a central role in the pathogenesis of many human malignancies.[23,33–36] Today known inherited mutations in the *CDKN2A* suppressor gene have conferred susceptibility to CMM and some other tumours such as oral, digestive system, breast, prostate, lung and bladder tumours as well as haematopoietic/lymphoproliferative system cancers.[3,8,37–42] It is presently unknown if patients with multiple CMM have a better prognosis than patients with single CMM.

Conclusion

Mutation carriers of the *CDKN2A* gene were younger when the first CMM was diagnosed and more often developed multiple CMM (group A). Neural tumours (no neurinoma of the acoustic nerve was found) occurred in a subgroup of young patients with multiple CMM (nonmutation carriers in group A) which might form a previously undescribed new syndrome. Single CMM occurred in two sets of late-onset disease: one group, mostly females, together with adenocarcinomas (group B); and another group with mainly NMSC (group C). Patients in group B and group C may harbour other, so far unknown, genetic defects and/or depend on interaction with different environmental risk factors, e.g. sun exposure. Further environmental or genetic studies of these subgroups might be of value and need to be confirmed in larger datasets and by others.

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