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A design for cancer case–control studies using only incident cases: experience with the GEM study of melanoma

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Background The population-based case–control study is not suited to the evaluation of rare genetic (or environmental) factors. The use of a novel case–control design in which cases have second primaries and controls are cancer survivors has been proposed for this purpose.

Methods We report results from an international study of melanoma that involved population-based ascertainment of incident cases of second or subsequent primary melanoma as the 'case' group and incident cases of first primary melanoma as the 'control' group. We evaluate the validity of the study design by comparing the results obtained for phenotypic factors that have been shown consistently to be associated with melanoma in previous conventional studies with the results from a conventional case–control study conducted in Connecticut and from literature reviews.

Results All but one of the known risk factors for melanoma were shown to be significantly associated with melanoma in our study, though the individual odds ratios appear to be somewhat attenuated relative to the magnitudes typically observed in the literature.

Conclusions Patients with a second or subsequent primary cancer of a single type represent a potentially valuable and under-utilized resource for the study of cancer aetiology.

Keywords Case-control study, case-only study, melanoma

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For decades the case–control study has been by far the most widely used design for identifying disease risk factors. It has been especially commonly used in cancer, the disease that prompted its initial methodological development.[1] In recent years epidemiological research has increasingly included assessment of genetic susceptibility. Furthermore, the genetic variants that have been shown most convincingly to be associated with cancer risk occur with low frequency. These genetic variants have generally been identified through linkage studies of high-risk families.[2] While a collection of multiple case families is optimal for identifying rare, high penetrance, disease-causing genetic variants, it is a poor resource for investigating the impact of risk factors in the general population, largely owing to the unknowable ascertainment biases that affect studies of multiple case families. Ideally these rare variants should be studied in a population-based

framework, but the conventional case–control approach is not attractive for studying risk factors that occur with low frequency, since the risk factor may only be observed in very few, if any, population-based controls unless the sample size is prohibitively large.

To circumvent this problem we developed a novel case–control design based on the recruitment of incident multiple and single primary cases of cancer, and implemented a version of this design in a prototype study of melanoma, the GEM (Genes, Environment and Melanoma) Study. In this report we describe the rationale for the GEM study design and our experiences in conducting the study. Since all participants are cancer patients, population-based identification of both cases and controls is relatively straightforward, in contrast to the conventional case–control study, where identification and recruitment of appropriate population-based controls can be challenging, especially in circumstances where the study base can only be accessed by random digit dialing.[3]

The most compelling rationale for this case-only design is statistical efficiency. When we wish to study a rare risk factor that confers a potentially high relative risk, such as a high penetrance genetic variant, one can hypothesize that the risk factor is considerably more common among cases with first primaries than (conventional) controls and also much more common among second primaries than among cases with a single primary. For a rare risk factor, these higher prevalences translate directly into increased statistical power compared with a conventional case–control study.[4] This was the primary motivation for the GEM study, where a major objective was to examine the frequencies of occurrence and relative risks for melanoma of germ-line variants in the CDKN2A gene, believed to occur in considerably $<1\%$ of melanoma cases, and by inference in a much smaller proportion of healthy population controls.[5]

The GEM study was also designed to study common genetic variants and known melanoma risk factors. The purpose of this article is to describe our practical experiences in conducting the GEM study, to evaluate its ability to identify known phenotypic risk factors, and to compare the relative risk estimates we obtained for these risk factors with the estimates that have been obtained in previous conventional melanoma case–control studies.

Methods

The GEM population consisted of incident cases of melanoma identified in eight population-based registries and one hospital centre (that sees ~50% of the melanomas diagnosed in the state of Michigan) in nine geographic regions of the world: New South Wales (Australia); Tasmania (Australia); British Columbia (Canada); Ontario (Canada); Turin (Piemonte, Italy); California (Orange County and San Diego/Imperial Organization for Cancer Control, USA); Michigan (USA); New Jersey (USA); and North Carolina (USA). The study was coordinated at the Memorial Sloan-Kettering Cancer Center (MSKCC).

Case and control ascertainment

GEM Controls were individuals diagnosed with a first invasive primary melanoma during the 6 month period January 1, 2000–June 30, 2000 with the following exceptions: the whole of 2000 in California, Michigan, and North Carolina, from January 1, 2000 to August 31, 2000 in Ontario, and from June 1, 2000 to May 31, 2001 in Turin, Italy. GEM Cases were individuals diagnosed with a second or higher order invasive or *in situ* melanoma during the period January 1, 2000 to August 31, 2003, except in Ontario where case ascertainment ended February 28, 2003, and the centres in British Columbia, California, New Jersey, and Tasmania, which recruited GEM Cases additionally in 1998 and 1999. A recurrence or metastasis from the initial melanoma did not qualify a subject as a case. All subjects were required to conduct a telephone interview in English (or Italian in Turin), contribute a DNA sample, and sign the informed consent. Central pathology review by a team of pathologists with expertise in melanoma was conducted for all subjects in the study for whom slides could be obtained.

Data collection

The study protocol was approved by the Institutional Review Boards at each participating institution. Physician approval was obtained before subject contact. After obtaining consent research staff mailed 4–6 buccal swabs for DNA analysis and a self-administered questionnaire to participants. Subsequently, a 1 h telephone interview was conducted in which further detailed information on lifetime ultraviolet radiation exposure and other variables was obtained. The self-administered questionnaire elicited date of birth, sex, hair colour as a teenager, eye colour, skin colour without tanning, freckling pattern in childhood, total body naevus pattern, skin sensitivity to sun exposure, and details of the occurrence of melanoma in all first-degree relatives. In addition to the above questions, subjects were asked to have the naevi on their backs counted. A coloured aid was included to assist them in differentiating between naevi and other skin lesions, such as freckles and seborrhoeic keratoses. Characteristics of melanomas, including anatomic site and Breslow thickness, were obtained from pathology reports.

Rationale and study power

In this article we focus our analysis on known phenotypic risk factors that can be used for comparison with conventional case–control studies, and family history of melanoma, although the design was motivated by the goal of evaluating the relative risk conferred by a germline CDKN2A mutation. The increased statistical efficiency of the design for this purpose can be illustrated as follows. We assumed that mutations would occur at a frequency of ~1% in incident melanoma cases (GEM Controls). We further assumed that the relative risk of melanoma for presence of a mutation might be in the region of 10. This seemed reasonable in view of the strong reported clustering of melanomas and high penetrance reported in CDKN2A-linked melanoma families.[6–8] Based on a projected accrual of 3000 GEM Controls and 800 GEM Cases we expected to observe 30 carriers among the 3000 GEM Controls and 74 carriers among the 800 GEM Cases. An analogous conventional study would be expected to identify only 3 carriers among 3000 healthy population controls, and 8 carriers among 800 incident cases. Thus, one would need almost 10 times as many participants in the conventional case–control study to achieve an equivalent statistical power.

Practical considerations

The closest replication of the conventional case–control study in this setting would be to select patients with incident second primary melanoma as cases, and to identify survivors of a first primary melanoma as controls, with the option to match on such factors as age, sex, and time since first primary diagnosis, for example. We chose the simpler strategy of recruiting incident first primary cases rather than 'prevalent' melanoma survivors as controls. Thus we included all incident cases in a defined accrual period, without selection, and sacrificed the option of stratifying cases and controls on the calendar time of occurrence of the first primary. Second, rather than restricting case accrual to second primary melanoma, we included as GEM Cases any patient with an incident second or higher-order melanoma during the case accrual period. This logistically convenient step allowed us greater access to the patients with the highest risk, i.e. those with multiple primaries, and also increased the rate of GEM Case accrual. Third, we decided to include a melanoma case as eligible if the second or subsequent primary was an *in situ* lesion. We did this because of our belief that the intense dermatological surveillance of melanoma survivors would cause *in situ* lesions to be identified and removed in many patients, leading to loss of participants who would have become GEM Cases without this intervention.

Critical assumption

The study design is based on a pivotal assumption: A second or subsequent primary occurring in a single individual is biologically independent of the first primary. That is, the cells in the second primary are not from the same clone of cells as the first primary, as would be the case, for example, in a metastasis. Our study relies on contemporary standards of pathological review to determine that second or subsequent primaries are not metastases, though we present data additionally on site concordance to shed further light on this important issue.

Statistical methods

We used standard analytic methods for case–control studies, comparing cases and controls using logistic regression to estimate odds ratios for individual risk factors, adjusted for other relevant risk factors. We addressed the likely impact of features of our strategy for sampling cases and controls in several ways. Our use of incident first primaries rather than prevalent survivors of melanoma as GEM Controls could lead to 'survival bias' if the risk factor under investigation is associated with case survival. We addressed this by comparing in patients with and without the risk factor the times from first to second primary diagnosis among GEM Cases diagnosed with a second primary. If the risk factor decreases survival following diagnosis then these times will be shorter on average for GEM Cases with the risk factor than for those without it and vice versa. We used a standard two-sample non-parametric Wilcoxon test for this purpose. We examined the impact of our inclusion as GEM Cases those who had multiple melanomas subsequent to the first by comparing the results with the results from an analysis in which the GEM Cases are restricted to incident second primaries. To examine the impact of our decision to include individuals with *in situ* lesions as GEM Cases, we examined the effect of an analysis restricted to invasive cases. To evaluate the possibility that some anatomically adjacent second primaries might be clonal products of the first primary we performed our analyses using only GEM Cases with primaries from anatomically distinct sites. A further unusual feature of the study is the fact that a considerable number of patients qualified as both a GEM Case and a GEM Control, i.e. they developed their first primary in the control accession period and their second primary in the case accession period. Epidemiological theory clearly indicates that these subjects should be included as both cases and controls in the analysis.[9] In our study 8% of the cases were crossovers.

Validation

Our premise in conducting the GEM study was that we would be reassured about the validity of our analyses of novel genetic risk factors if we could reproduce in this study the associations of melanoma with standard risk factors that have been consistently found in previous conventional case–control studies. Therefore, in this report we focus our attention on known risk factors: sex, melanocytic naevus (mole) count, freckling in childhood, burning and tanning ability, hair colour, eye colour, and family history of melanoma, and we evaluate the validity of the design empirically. That is, we compare our results with a previous conventional population-based case–control study led by one of the authors (M.B.).[10] This study involved a comparison of 650 incident cases of cutaneous melanoma recruited between 1987 and 1989 in the State of Connecticut with 549 population controls, obtained by random digit dialling, and frequency matched by age and sex. We also compare our results with the results from four meta-analyses, two concerning the risks associated with aspects of complexion,[11,12] one concerning the influence of common naevi,[13] and two addressing family history of melanoma.[13,14]

Results

A total of 4560 individuals were ascertained as eligible GEM Controls, of which 2470 (54%) participated. A corresponding total of 2308 individuals were ascertained as GEM Cases of which 1210 (52%) participated. Ninety-six subjects participated as both GEM Cases and GEM Controls. Participants were those subjects who completed the questionnaire and supplied a buccal or blood sample. The participation rates ranged from a low of 40% in New Jersey to a high of 82% in Tasmania. Women were slightly more likely to participate than men (56% vs 52%) and the mean ages were similar in both groups (58 in participants vs 60 in refusers). The principal reasons for non-participation were patient refusal, early death, physician refusal, or inability to make contact or loss of contact with the patient. Central pathology review has been obtained to date for 69% of the participants.

The distributions of age and sex are shown in Table 1. GEM Cases were generally older than GEM Controls, and the female/male ratio declines with age, reflecting the higher rate of increase in melanoma incidence with age in men than in women. These facts necessitate stratification for age,

Table 1 Age and sex

	GEM Controls		GEM Cases	
Age	Female	Male	Female	Male
<30	93 (8%)	47 (4%)	13 (3%)	8 (1%)
30–49	485 (41%)	292 (23%)	89 (22%)	60 (8%)
50–69	392 (33%)	592 (46%)	183 (44%)	343 (43%)
⩾70	224 (19%)	345 (27%)	127 (31%)	387 (48%)

sex, and age/sex interaction in our analyses of other risk factors.

The results of the logistic regression analysis of all the melanoma phenotypic risk factors are displayed in Table 2 alongside the corresponding results from the conventional Connecticut case–control study. The only risk factor that was statistically significantly associated with risk in the Connecticut study, but not in the GEM study, is eye colour. For the other risk factors the results are broadly similar, though the odds ratios in the GEM study were generally less than in the Connecticut study. It should be noted that the mole count in the Connecticut study involved all moles on the back and arms, counted by trained nurses, while in the GEM study the mole count is for the back only and was self-reported.

We also compared the results with those from available meta-analyses of individual risk factors. The results are displayed in Table 3. Two of these examined 'complexion' factors.[11,12] The GEM results for hair colour are consistent with the meta-analysis, though somewhat attenuated. The meta-analysis indicated an association with eye colour that is not replicated in either the GEM study or the Connecticut case–control study. In their meta-analysis of the role of naevi, Gandini *et al*.[12] summarize results in terms of whole-body counts of common naevi (six categories), and counts on the arms alone (four categories). Although neither of these is directly comparable with the back mole count used in GEM, we have compared them with our results by simply creating six and four categories of mole count, respectively, with equal frequencies of subjects in each category. The GEM odds ratios for naevus counts were less than those of the meta-analysis. The relative risk for family history in GEM is of a similar magnitude to that obtained in both the Connecticut study (Table 2) and the two meta-analyses of this issue (Table 3).

The inclusion of *in situ* melanomas as GEM Cases made the Breslow depths of these subsequent primaries significantly less than those of GEM Controls (Table 4), and when this comparison is limited to invasive GEM Cases there is still a higher proportion (76%) with lesions <1 mm thick than in GEM Controls (69%). However, the distribution of tumour depths in the first primary for GEM Cases was similar to the distribution in GEM Controls. When we compare the analysis using all GEM Cases with an analysis in which the *in situ* cases are removed (third and fourth columns of Table 5) we see only a very slight strengthening of the results. When we restrict our GEM Cases to only those with an incident second primary during the case accession period there is very little difference in the odds ratios (third vs fifth columns of Table 5). When we compare this analysis with one in which multiple primary GEM Cases represent the case group and second primary cases represent the control group (last column of Table 5) we see the trends in all the odds ratios replicated, as we would expect, though apparently further attenuated.

Table 2 Results comparison with conventional case–control study[a]

Factor	Level	GEM[b]	Connecticut C-C Study
Sex and age	Age < 50: female vs male	0.9 (0.6–1.3)	Matched
Interaction	Age 50–69: female vs male	0.7 (0.3–1.7)	
	Age ⩾ 70: female vs male	0.5 (0.2–1.8)	
Moles[c]	0	1.0	1.0
	⩽10	1.3 (1.0–1.7)	2.0 (1.3–3.3)
	11–30	1.8 (1.4–2.4)	5.1 (3.0–8.5)
	31–50	2.7 (1.9–4.0)	8.0 (3.9–16.3)
	>50	2.9 (2.0–4.2)	7.0 (3.2–15.3)
Freckles in childhood	No	1.0	1.0
	Yes	1.3 (1.1–1.6)	1.4 (1.0–1.9)
Propensity to burn	No	1.0	1.0
	Yes	1.1 (0.9–1.3)	0.9 (0.7–1.3)
Propensity to tan	Yes	1.0	1.0
	No	1.2 (1.0–1.5)	2.0 (1.4–2.9)
Hair colour	Dark	1.0	1.0
	Light	1.4 (1.2–1.7)	1.6 (1.1–2.1)
Eye colour	Dark	1.0	1.0
	Light	0.9 (0.7–1.1)	1.5 (1.1–2.1)
Family history of melanoma	No	1.0	1.0
	Yes	1.7 (1.4-2.2)	1.8 (1.2-2.7)

[a] Results show odds ratios and 95% confidence intervals from a logistic regression analysis in which all listed factors and age and sex are included in the model.

[b] Adjusted for age, sex, and centre in addition to all other variables in the table.

[c] Moles counted in GEM study on back only, in Connecticut Study on back and arms.

In Table 6 we display the site concordance of the two index lesions among GEM Cases for whom body site was specified with sufficient detail for both lesions (98% of total). There is modest site concordance (kappa = 0.15), with 340 (29%) of the cases having the two lesions in the same general anatomic region, vs 191 (16%) expected. We performed the case–control analysis after removing the 340 GEM Cases with concordant disease sites (column 6 of Table 5) and we observe only small differences in the results when compared with the analysis involving all GEM Cases.

We used the times from first to second primary diagnosis among GEM Cases with second primaries to test for survival bias. The comparisons for all factors were not significant, with the exception of gender, with the mean durations being significantly longer for women than for men (5.6 vs 4.7 years, $P < 0.01$). This may reflect the fact that incident melanoma cases tend to occur at younger ages in women than in

Table 3 Results comparison with recent meta-analyses[a]

Factor	Level	GEM[a]	Meta–analyses	
Hair colour	Black/dark brown	1.0	1.0[b]	1.0[c]
	Light brown	1.3 (1.1–1.7)	1.5 (1.3–1.7)	1.6 (1.1–2.3)
	Blonde	1.3 (1.0–1.6)	1.8 (1.5–2.2)	2.0 (1.4–2.7)
	Red	2.0 (1.5–2.8)	2.4 (1.9–3.0)	3.6 (2.6–5.4)
Eye colour	Brown	1.0	1.0[b]	1.0[c]
	Green/grey/hazel	1.0 (0.8–1.2)	1.3 (1.1–1.5)	
	Blue	1.0 (0.8–1.3)	1.5 (1.3–1.8)	1.5 (1.3–1.8)
Moles (whole body)	0–15	1.0	1.0[d]	
	16–40	1.4 (1.1–1.8)	1.5 (1.4–1.6)	
	41–60	1.4 (1.0–1.8)	2.2 (1.9–2.6)	
	61–80	1.6 (1.2–2.1)	3.3 (2.6–4.2)	
	81–100	2.0 (1.5–2.7)	4.7 (3.4–6.5)	
	101–120	2.8 (2.1–3.7)	6.9 (4.6–10.3)	
Moles (arms)	0	1.0	1.0[d]	
	1–5	1.4 (1.4–1.8)	1.4 (1.3–1.6)	
	5–10	1.7 (1.3–2.1)	2.5 (1.9–3.2)	
	11–15	2.5 (2.0–3.2)	4.8 (3.1–7.6)	
Family history	No	1.0	1.0[e]	1.0[f]
	Yes	1.7 (1.4–2.2)	2.2 (1.8–2.9)	1.7 (1.4–2.1)

[a] Odds ratios based on a logistic regression including age, sex, age/sex interaction, mole count, freckling in childhood, propensity to burn, propensity to tan, and centre.

[b] From Bliss *et al.*[11]

[c] From Gandini *et al.*[12]

[d] From Gandini *et al.*[13]

[e] From Ford *et al.*[14]

[f] From Gandini *et al.*[12]

men (Table 1), resulting in fewer deaths from other causes, although it may also reflect a genuine difference in case survival.[15]

Discussion

The results of this study show that using our novel study design the known risk factors for melanoma were identified with convincing statistical significance (except for eye colour), though with observed relative risks that seem to be of a somewhat smaller magnitude than the estimates from conventional case–control studies. Our multi-institutional study was able to identify large numbers of participants in a reasonable time frame. Participation rates of 54 and 52% for GEM Controls and GEM Cases, respectively, were lower than anticipated (Table 7). The principal reason for non-participation was patient refusal, which probably reflects a general trend towards a lowered willingness for patients to participate as volunteers in research studies, especially those for which biological specimens are required, although such trends are difficult to discern owing to inconsistent metrics for reporting non-participation.[16,17] The fact that the participation rates are

Table 4 Breslow depth comparison

Breslow depth (mm)	GEM Controls (%)	GEM Cases Most recent primary (%)	GEM Cases Previous primary (%)
0 (*in situ*)	0	37	0
<1	69	48	69
1–2	19	9	18
2–4	8	4	10
>4	4	2	3

virtually identical for cases and controls is reassuring, since it is differential recruitment patterns in the two groups that are most likely to lead to bias.

The directionality and statistical significance of the relative risks we observed are consistent with those obtained in conventional case–control studies, but they do appear to be attenuated. Our study design involves the case–control evaluation of risk in a population (melanoma survivors) whose members have a markedly high 'background' risk of the disease. Thus, we can expect similar relative risk magnitudes only if the relative risks of common risk factors are the same in populations with high background risk as in the general population. Unfortunately, if attenuation of relative risks proves to be commonplace in this setting, this will reduce the degree by which statistical power is enhanced.

We elected to use incident sampling for both GEM Cases and GEM Controls, both for convenience, and to permit a direct population-based interpretation of the frequencies observed for both groups. However, other design options are possible. For example, we could have pair-matched the cases and controls to increase efficiency or we could have selected prevalent controls and matched on the date of diagnosis of the first primary. Further study of these and other design options will help to refine the utility of the design.

The pivotal assumption in our study design is that the multiple melanomas observed in an individual patient are biologically independent. Genetic susceptibility and exposure to environmental risks lead to clustering of melanomas in individual subjects, but our design is based on the premise that, conditional on these risks, the occurrences of individual melanomas in the same patient are statistically independent (i.e. conditionally independent). This assumption is violated if the multiple primaries are clonal products of a single cell that has experienced one or more somatic mutations. The degree of clonality of 'independent' primary cancers has been examined in numerous studies in recent years using mutational profiling of genes that occur frequently in tumours, such as p53, or loss of heterozygosity of selected markers. If the patterns of the variants in the tumours are similar then they are considered to be clonal. In general, these studies have shown that clonality is quite common for mucosal cancers of the head and neck,[18] and bladder.[19] However, for sites with paired contralateral organs, such as breast[20–23] and lung,[24–26] the vast majority of new primaries appear to be biologically independent. The issue does not appear to have been studied in melanoma, but the wide anatomic distribution of melanomas and the absence of a plausible mechanism for the seeding of clonal cells in distant parts of the skin argue against the frequent clonality of multiple primaries in this disease. Also, our re-analysis eliminating GEM

Table 5 Alternative analytic approaches[a]

		GEM Cases				
Factor	**Level**	All cases (n = 3680)	Invasive cases only (n = 3252)	2nd Primary cases only (n = 3402)	Discordant cases only[b] (n = 3340)	Higher order compared with 2nd primary cases (n = 1299)
Sex and age interaction	Age < 50: female vs male	0.9 (0.6–1.3)	0.8 (0.5–1.3)	0.9 (0.6–1.3)	1.1 (0.7–1.8)	1.3 (0.6–2.9)
	Age 50–69: female vs male	0.7 (0.3–1.7)	0.7 (0.3–1.8)	0.8 (0.3–1.9)	0.8 (0.3–2.3)	0.8 (0.1–4.4)
	Age ⩾ 70: female vs male	0.5 (0.2–1.8)	0.5 (0.2–1.5)	0.5 (0.2–1.3)	0.6 (0.2–1.6)	0.8 (0.1–4.9)
Moles (back)	0	1.0	1.0	1.0	1.0	1.0
	⩽10	1.3 (1.0–1.7)	1.5 (1.1–2.0)	1.3 (1.0–1.7)	1.4 (1.1–1.9)	1.2 (0.8–1.9)
	11–30	1.8 (1.4–2.4)	1.8 (1.3–2.5)	1.8 (1.3–2.3)	1.9 (1.4–2.6)	1.4 (0.9–2.2)
	31–50	2.7 (1.9–4.0)	3.0 (2.0–4.7)	2.6 (1.7–3.8)	2.8 (1.9–4.3)	1.3 (0.7–2.5)
	>50	2.9 (2.0–4.2)	3.3 (2.2–5.0)	2.6 (1.7–3.8)	2.8 (1.8–4.2)	1.9 (1.0–3.4)
Freckles in childhood	No	1.0	1.0	1.0	1.0	1.0
	Yes	1.3 (1.1–1.6)	1.2 (1.0–1.5)	1.2 (1.0–1.5)	1.2 (1.0–1.5)	1.2 (0.9–1.6)
Propensity to burn	No	1.0	1.0	1.0	1.0	1.0
	Yes	1.1 (0.9–1.3)	1.1 (0.9–1.4)	1.1 (0.9–1.3)	1.1 (0.9–1.4)	1.0 (0.8–1.3)
Propensity to tan	Yes	1.0	1.0	1.0	1.0	1.0
	No	1.2 (1.0–1.5)	1.2 (1.0–1.5)	1.2 (1.0–1.5)	1.2 (1.0–1.5)	1.3 (0.9–1.7)
Hair colour	Dark	1.0	1.0	1.0	1.0	1.0
	Light	1.4 (1.2–1.7)	1.6 (1.2–2.0)	1.4 (1.1–1.7)	1.4 (1.2–1.8)	1.2 (0.9–1.7)
Eye colour	Dark	1.0	1.0	1.0	1.0	1.0
	Light	0.9 (0.7–1.1)	0.9 (0.7–1.1)	0.9 (0.7–1.2)	0.9 (0.7–1.1)	0.8 (0.5–1.1)
Family history	No	1.0	1.0	1.0	1.0	1.0
	Yes	1.7 (1.4–2.2)	1.9 (1.5–2.5)	1.6 (1.3–2.0)	1.7 (1.4–2.2)	1.5 (1.1–2.1)

[a] Results show odds ratios and 95% confidence intervals from logistic regression analyses in which all listed factors and age, sex, and centre are included in the model.

[b] GEM Cases with concordant anatomic sites of the two index lesions are excluded from this analysis.

Cases with anatomically adjacent primaries had little impact on the estimated odds ratios.

Melanoma is an especially attractive candidate for a case–control study of this type, since second primaries occur frequently in this disease. Current medical treatments are not especially effective. In contrast, when this design is used in breast cancer, adjuvant treatment for the first primary, using tamoxifen or chemotherapy, is known to influence the occurrence of second (contralateral) primaries, and so one would have to be cautious in interpreting relative risks of factors that may interact with treatment. A large study of this nature is currently in progress, and the results will be informative with regard to this issue.[27] In our study we have restricted attention to phenotypic factors and family history. The impact of the design in evaluating the major environmental risk factor, sun exposure, will be examined in detail in another article. For environmental factors one needs to be concerned that behaviour changes stimulated by the initial melanoma diagnosis may subsequently alter the risk status and thus influence the relative risks obtained from this design. Indeed, in the case of sun exposure evidence has emerged recently that this factor may influence survival from melanoma, and this has potential to cause bias in the relative risk estimates.[28]

There is a rich history of research on the incidence and characteristics of second primary cancers.[29] Much of this research has used cohorts identified from cancer registries to examine the influence of cancer treatments on the risk of subsequent primaries, notably radiotherapy and specific chemotherapeutic agents.[30–35] *De novo* analytic epidemiological studies of second primaries have been rare, though there are a few exceptions.[36–38] Even so, these have usually been conducted with the purpose of identifying risk factors for second cancers specifically, rather than as an indirect means of uncovering facts about the incidence of the cancer in general, i.e. risk factors for first primaries. The aging of the population has resulted in many more cancer survivors in the population and many more occurrences of second primaries. We believe that multiple primary cancers represent a potentially valuable and relatively untouched resource for the study of cancer aetiology, especially for the study of rare risk factors. The design would seem to be attractive in sites where clonality of second primaries is unlikely, such as breast, lung, and melanoma.

Table 6 Concordance of body sites of observed melanomas in GEM Cases[a]

	Site of melanoma (Case defining)																	
	Head/neck					Trunk				Arms				Legs				
Site of melanoma (previous)	Scalp	Face	Neck	Ear	Unspc.	Pstr.	Antr.	Pelvis	Unspc.	Upper	Lower	Hand	Unspc.	Upper	Lower	Foot	Unspc.	**Total**
Head/neck																		
Scalp	5	4	1	2	0	6	2	0	0	1	2	0	0	0	1	0	1	25
Face	5	37	2	4	0	21	1	0	1	7	5	0	2	4	0	0	1	90
Neck	2	3	5	1	0	11	2	1	0	3	3	0	1	1	2	0	2	37
Ear	1	6	3	4	0	8	3	1	1	1	2	0	0	1	2	0	1	34
Unspc.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Trunk																		
Pstr.	14	37	10	7	1	213	20	1	2	29	21	0	12	17	27	0	6	417
Antr.	4	13	6	0	0	38	11	1	2	9	3	0	1	6	5	0	3	102
Pelvis	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	2
Unspc.	0	6	1	3	1	5	0	0	1	3	1	0	4	1	1	0	2	29
Arms																		
Upper	3	9	4	1	1	25	3	0	2	8	5	0	2	4	9	1	1	78
Lower	3	13	5	1	0	20	1	1	0	5	7	0	3	5	5	0	1	70
Hand	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1	6
Unspc.	3	9	1	2	1	12	5	1	5	4	2	1	9	4	3	0	4	66
Legs																		
Upper	2	7	4	3	0	18	4	0	1	5	5	0	2	16	11	0	2	80
Lower	0	9	5	1	0	23	4	1	0	8	6	1	8	11	21	4	2	104
Foot	2	0	0	0	0	5	1	0	0	1	1	0	0	2	1	1	1	15
Unspc.	1	3	1	1	0	3	3	0	1	2	0	0	5	4	5	0	2	31
Total	45	157	49	30	4	409	61	7	16	87	64	2	50	76	93	7	30	1187

[a] These results are restricted to GEM Cases and classify the site of occurrence of the index (case defining) second of higher order primary that qualified the subject as a GEM Case (horizontal axis) with the site of the previous melanoma (vertical axis). Information on both sites was available for 98% (1187/1210) of the GEM Cases.

Table 7 Recruitment of patients[a]

	Controls	Cases
Eligible and approached[b]	4560	2308
Non-participants	2090	1098
Deceased prior to contact	170	94
Physician refusal	172	92
Patient refusal	1330	644
Lost to follow-up	418	268
Participated/Analysed	2470 (54%)	1210 (52%)

[a] There are 96 patients in the dataset who belong to both the case and the control group. Thus the total number of analysed participants is 3584.

[b] 346 controls and 261 cases were ascertained but found to be ineligible on review of pathology reports (n = 182 controls, 196 cases) or they were unable to complete a telephone interview because they were deaf or mute (n = 55 controls, 23 cases), dementia or in nursing home (n = 68 controls, 33 cases) non-English speaking (n = 31 controls, 7 cases) or unaware of diagnosis (n = 10 controls, 2 cases).

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KEY MESSAGES

- Comparison of an incident series of second primary cancers with incident first primaries is a viable strategy for conducting population-based case-control studies.
- There may be a tendency for relative risks of individual risk factors to be attenuated in populations of high baseline risk.

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