An Assessment of a Variant of the DNA Repair Gene XRCC3 as a Possible Nevus or Melanoma Susceptibility Genotype

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Inheritance of the T allele in exon 7 (position 18067) of the DNA repair gene XRCC3 has been reported to be associated with susceptibility to melanoma in a study from Oxford. We report a study in which an attempt was made to confirm this association in a similar population. The most potent risk factor for melanoma in the general population is a phenotype characterized by the presence of multiple melanocytic nevi: the atypical mole syndrome. Our hypothesis is that the atypical mole syndrome may be a marker of genetic susceptibility to melanoma. We have therefore investigated whether the XRCC3 polymorphism influences the nevus phenotype. The XRCC3 genotype was investigated using PCR in a general-practice-based sample of 565 women and 475 patients from a cohort enriched for the atypical mole syndrome, of whom 140 had had melanoma. Allele frequencies were the same in the healthy women, the melanoma cases from this study, and the melanoma cases reported in the Oxford study, but were different from those in the Oxford control group. We found no evidence therefore that the T allele of this XRCC3 polymorphism is indicative of susceptibility to melanoma. There was a marginal relationship with nevus phenotype, but this was no longer statistically significant in multivariate analysis. The previous association between XRCC3 and melanoma may be a result of the choice of control group and we emphasize the need for appropriate choice of controls.

Key words: DNA repair/melanoma/nevi/stroke/XRCC3.
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Inheritance of the T allele in exon 7 (position 18067) of the DNA X-ray repair cross-complementing gene 3 (XRCC3) has been reported to be associated with susceptibility to melanoma in a population from Oxford, UK ($p = 0.004$; odds ratio 2.36) (Winsey *et al*, 2000) although this was not confirmed in a US population (Duan *et al*, 2002). The functional significance of the polymorphism is not yet known. The most potent phenotypic risk factor for melanoma in the general population is the atypical mole syndrome (AMS) phenotype (Augustsson *et al*, 1990; Halpern *et al*, 1991; Bataille *et al*, 1996). Our hypothesis is that the AMS may be a marker of genetic susceptibility to melanoma: that nevus genes may be low penetrance melanoma susceptibility genes. We have therefore determined if the XRCC3 polymorphism influences the nevus phenotype in two populations.

Results

Genotype frequencies (Table I) showed no significant difference in distribution between the healthy women and the AN/FM group ($\chi^2 = 0.98$ with two degrees of freedom, $p = 0.61$). The T allele frequency was similar in the healthy women, the AN/FM group who had melanoma, and in those who did not. All groups were in Hardy–Weinberg equilibrium.

In the AN/FM group the univariate analysis showed a consistent increase in total number of nevi (p value for linear trend 0.02) and number of atypical nevi (p value for linear trend 0.05) with increasing numbers of T alleles (Table II). In multivariate analyses, however, there was no evidence of a relationship with genotype (Tables II, III) and the above trends were not significant ($p = 0.28$ and $p = 0.17$, respectively).

No associations were seen between either total or atypical nevus count and XRCC3 genotype, in univariate or multivariate analysis, amongst the GP group (Tables II, III).

Discussion

Allele frequencies were the same in the GP group, the melanoma cases from this study, and the melanoma cases reported in the Oxford study published earlier, but were different from those in the Oxford control group ($p = 0.001$) (Winsey *et al*, 2000). Therefore the disparity between our findings and those of the Oxford group arises as a result of a different prevalence of XRCC3 T alleles in the donors of

Abbreviations: AMS, atypical mole syndrome; AN/FM, study group recruited in abnormal nevus phenotype and familial melanoma research programme; XRCC3, X-ray repair cross-complementing gene 3.

Table I. Genotype frequencies in this study and in the studies reported by Duan *et al* (2002) and Winsey *et al* (2000)

	This study UK, AN/FM ($n = 475$)		This study UK controls (GP group) ($n = 565$)		Duan *et al* (2002), USA		Winsey *et al* (2000), Oxford	
	Patients without melanoma ($n = 335$)	Melanoma cases ($n = 140$)	Yorkshire ($n = 362$)	Hertfordshire ($n = 203$)	Cases ($n = 305$)	Controls ($n = 319$)	Oxford melanoma cases ($n = 125$)	Donor controls in the Oxford study ($n = 211$)
Mean age (sd)	40 (17.5)	48 (13.6)	36 (6.7)	37 (6.2)	49	51	52[a]	
Female percentage	55	61	100	100	48	49	42	
CC	135	50	140	69	119	116	39	110
n (%)	(40%)	(36%)	(39%)	(34%)	(39%)	(36%)	(31%)	(52%)
CT	160	68	170	101	148	158	65	78
n (%)	(48%)	(48%)	(47%)	(50%)	(48%)	(50%)	(52%)	(37%)
TT	40	22	52	33	38	45	21	23
n (%)	(12%)	(16%)	(14%)	(16%)	(13%)	(14%)	(17%)	(11%)
Frequency of T allele	36%	40%	38%	41%	37%	39%	43%	29%
Hardy–Weinberg[b]	$p = 0.48$	$p = 0.89$	$p = 0.97$	$p = 0.70$	$p = 0.44$	$p = 0.45$	$p = 0.49$	$p = 0.11$

The GP group were female and the AN/FM group were of both sexes.
[a]Median age.
[b]p value for test of departure from Hardy–Weinberg equilibrium.

Table II. Predictors of nevus phenotype: univariate analyses of nevus characteristics with genotype in both the AN/FM and the GP groups

	CC, mean (SD)	CT, mean (SD)	TT, mean (SD)	ANOVA, p value	Test for trend, p value
AN/FM group					
AMS 0–5 ($n = 475$)	($n = 185$)	($n = 228$)	($n = 62$)		
Log total nevus count	4.06 (1.20)	4.21 (1.20)	4.47 (1.19)	0.07	0.02
Log atypical nevus count	0.77 (0.99)	0.89 (1.02)	1.07 (1.08)	0.14	0.05
AMS score	1.89 (1.35)	2.11 (1.41)	2.31 (1.35)	0.09[a]	0.03[a]
AMS 2–5 ($n = 289$)	($n = 103$)	($n = 143$)	($n = 43$)		
Log total nevus count	4.86 (0.70)	4.88 (0.66)	5.02 (0.62)	0.40	0.25
Log atypical nevus count	1.27 (1.05)	1.32 (1.02)	1.42 (1.06)	0.72	0.44
Probands AMS 0–5 ($n = 174$)	($n = 58$)	($n = 90$)	($n = 26$)		
Log total nevus count	4.83 (0.92)	4.97 (0.86)	5.16 (0.60)	0.27	0.11
Log atypical nevus count	1.25 (1.06)	1.40 (1.05)	1.68 (0.96)	0.22	0.09
Probands AMS 2–5 ($n = 157$)	($n = 51$)	($n = 82$)	($n = 24$)		
Log total nevus count	5.07 (0.66)	5.15 (0.54)	5.26 (0.49)	0.39	0.17
Log atypical nevus count	1.35 (1.06)	1.52 (1.01)	1.79 (0.90)	0.22	0.09
GP group					
AMS 0–5 ($n = 565$)	($n = 209$)	($n = 271$)	($n = 85$)		
Log total nevus count	3.74 (0.96)	3.69 (0.86)	3.62 (0.86)	0.61	0.33
Log atypical nevus count	0.17 (0.42)	0.14 (0.36)	0.14 (0.39)	0.77	0.55
AMS 2–5 ($n = 107$)	($n = 50$)	($n = 43$)	($n = 14$)		
Log total nevus count	4.66 (0.60)	4.51 (0.61)	4.66 (0.66)	0.48	0.61
Log atypical nevus count	0.44 (0.65)	0.39 (0.60)	0.38 (0.68)	0.92	0.70

Nevus counts were log-transformed to reduce positive skew of the distributions.
[a]Ordinal regression.

Table III. Predictors of nevus phenotype: multivariate analyses of nevus characteristics with genotype in both the AN/FM and the GP groups

Parameters in the model	Coefficient (standard error)	p-value
Log total nevus count (AN/FM group)		
Age	0.07 (0.01)	<0.0001
Age-squared	−0.001 (0.0002)	<0.0001
Sex — females *versus* males	−0.07 (0.09)	0.44
Melanoma — present *versus* absent	0.61 (0.10)	<0.0001
XRCC3 — CT *versus* CC	0.09 (0.10)	0.35
XRCC3 — TT *versus* CC	0.16 (0.14)	0.27
Log atypical nevus count (AN/FM group)		
Age	0.03 (0.01)	0.05
Age-squared	−0.0004 (0.0001)	0.005
Sex — females *versus* males	−0.17 (0.08)	0.04
Melanoma — present *versus* absent	0.59 (0.10)	<0.0001
XRCC3 — CT *versus* CC	0.12 (0.09)	0.21
XRCC3 — TT *versus* CC	0.14 (0.14)	0.30
AMS score (AN/FM group)[a]		
Age	0.10 (0.03)	<0.0001
Age-squared	−0.001 (0.0003)	<0.0001
Sex — females *versus* males	−0.15 (0.17)	0.37
Melanoma — present *versus* absent	1.13 (0.18)	<0.0001
XRCC3 — CT *versus* CC	0.35 (0.19)	0.06
XRCC3 — TT *versus* CC	0.29 (0.26)	0.26
Log total nevus count (GP group)[b]		
XRCC3 — CT *versus* CC	−0.06 (0.08)	0.49
XRCC3 — TT *versus* CC	−0.12 (0.12)	0.31
Log atypical nevus count (GP group)[b]		
XRCC3 — CT *versus* CC	−0.03 (0.03)	0.35
XRCC3 — TT *versus* CC	−0.04 (0.05)	0.46

Nevus counts were log-transformed to reduce positive skew of the distributions. All analyses adjusted for examiner, and analyses of the AN/FM group included family as a random effect.

[a]Ordinal regression.

[b]In the GP group, analyses were adjusted for age and age squared as in the AN/FM group, although the relationships were in this case not significant.

organs used as controls. The cause of death in UK cadaveric donors, however, is nonrandom as the majority die from intracranial hemorrhage (NHS UK Transplant). It is not inconceivable that a DNA repair gene might play a role in susceptibility to early stroke (Kim *et al*, 2001; Goto *et al*, 2002). Spurious correlations between genotypes and disease may arise by chance, or as a result of population stratification (where disease and genotype are correlated in the population, due to confounding, generally by ethnic or regional differences in both allele frequencies and disease rates (Thomas and Witte, 2002; Wacholder *et al*, 2002)). There is also agreement that the practice of using convenience samples as controls may give rise to false positive associations, although the practice remains widespread. Comparisons between our sampled groups are subject to the criticisms outlined above, but the same potential for bias does not exist within the two studies as our controls were not selected by disease. The fact that the subjects without melanoma are selected on the basis of nevus phenotype would tend to minimize any differences in allele frequencies for a true melanoma/nevus gene. Nonetheless we believe that the results provide strong evidence that the original association was spurious. Within the UK, two studies have shown similar allele frequencies in two sets of melanoma cases but different frequencies in the controls. Furthermore the allele frequencies in our two UK control groups (Yorkshire and Hertfordshire) and in the US controls (Duan *et al*, 2002) were similar. It therefore seems most likely that the Oxford controls, rather than the healthy women studied here, are unrepresentative of the normal population. The issue of control selection remains a critical but contentious one.

Our study found no evidence that the T allele of this XRCC3 polymorphism is indicative of susceptibility to an atypical nevus phenotype. We did find a marginal relationship between inheritance of the T allele and AMS score, the number of nevi, and the number of atypical nevi (Table II) but no significant association once appropriate corrections for age, sex, and familial clustering were made (Table III). It is not possible to exclude the possibility that there is a real relationship between nevi and XRCC3, which would require a larger study to explore.

Methods

565 white women were recruited from the general population via general practices in Yorkshire and Hertfordshire (GP group). 475 white subjects, of whom 140 had had melanoma, consisting of 174 probands and their adult relatives, were studied from a cohort enriched for the AMS as described previously (Bertram *et al*, 2002) (atypical nevi/familial melanoma (AN/FM) group). Ethical approval was obtained from local ethics committees prior to data collection.

Banal and atypical nevi were counted as previously described, and the AMS score was computed (Bertram *et al*, 2002). DNA was extracted from blood, and the XRCC3 polymorphism was detected using PCR as described previously (Winsey *et al*, 2000).

Statistical analysis was performed using the statistical package Stata (StataCorp, College Station, TX).

Primary analyses were based on the three XRCC3 genotypes (CC, CT, TT) and secondary analyses examined trend. Associations between nevus counts and XRCC3 genotype were analyzed using analysis of variance and tests for linear trend. In the AN/FM group, ordinal regression was used to estimate associations between XRCC3 genotype and AMS score. Analyses were repeated amongst probands only and in subjects with an abnormal phenotype (AMS score 2–5).

Multivariate regression was also used, adjusting for age, sex (AN/FM only), melanoma status (AN/FM only), and examiner. The AN/FM analyses also took account of familial clustering by using random effects models.

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References

Augustsson A, Stierner U, Rosdahl I, Suurkula M: Common and dysplastic naevi as risk factors for cutaneous malignant melanoma in a Swedish population. Acta Derm Venereol 71:518–524, 1990

Bataille V, Bishop JA, Sasieni P, Swerdlow AJ, Pinney E, Griffiths K, Cuzick J: Risk of cutaneous melanoma in relation to the numbers, types and sites of naevi: A case-control study. Br J Cancer 73:1605–1611, 1996

Bertram CG, Gaut RM, Barrett JH, *et al*: An assessment of the CDKN2A variant Ala148Thr as a nevus/melanoma susceptibility allele. J Invest Dermatol 119:961–965, 2002

Duan Z, Shen H, Lee JE, *et al*: DNA repair gene XRCC3 241Met variant is not associated with risk of cutaneous malignant melanoma. Cancer Epidemiol Biomarkers Prev 11 (10 Part 1):1142–1143, 2002

Goto S, Xue R, Sugo N, *et al*: Poly (ADP-ribose) polymerase impairs early and long-term experimental stroke recovery. Stroke 33:1101–1106, 2002

Halpern AC, Guerry DP IV, Elder DE, Clark WH Jr, Synnestvedt M, Norman S, Ayerle R: Dysplastic nevi as risk markers of sporadic (nonfamilial) melanoma. Arch Dermatol 127:995–999, 1991

Kim GW, Noshita N, Sugawara T, Chan PH: Early decrease in dna repair proteins, Ku70 and Ku86, and subsequent DNA fragmentation after transient focal cerebral ischemia in mice. Stroke 32:1401–1407, 2001

Thomas D, Witte J: Point: Population stratification: A problem for case-control studies of candidate–gene associations? Cancer Epidemiol, Biomarkers Prevention 11:505–512, 2002

Wacholder S, Rothman N, Caporaso N: Counterpoint: Bias from population stratification is not a major threat to the validity of conclusions from epidemiological studies of common polymorphisms and cancer. Cancer Epidemiol Biomarkers Prev 11:513–520, 2002

Winsey SL, Haldar NA, Marsh HP, *et al*: A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. Cancer Res 60:5612–5616, 2000