ORIGINAL ARTICLE

Non-genetic and genetic predictors of a superficial first basal cell carcinoma

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Abstract

Background Several observational studies have suggested differences in the risk factor profile between patients with superficial basal cell carcinomas (BCCs) and non-superficial BCCs.

Objective To test the reproducibility of previous study findings and to find new genetic and non-genetic predictors for patients with a superficial first BCC.

Methods A total of 14.628 participants of northwestern European descent aged 45 years or older from a prospective population-based cohort study (Rotterdam Study) were linked with the Dutch Pathology Registry (PALGA) of whom 1528 were identified as BCC patients. After exclusion, 948 eligible BCC patients remained for further non-genetic analyses and 1014 for genetic analyses. We included 11 phenotypic, environmental and tumour-specific characteristics, and 20 candidate single nucleotide polymorphisms (SNP) as potential predictors for patients with a superficial first BCC. We performed binary logistic multivariable regression analyses.

Results We found that patients with a superficial first BCC were significantly younger, almost two times more often female and 12–18 times more likely to have their BCC on the trunk or extremities than patients with a non-superficial first BCC. One SNP (rs12203592), mapped to IRF4, looked promising (OR 1.83, 95% CI 1.13–2.97, *P*-value <0.05), but after adjustment for multiple testing, no significant differences in genetic make-up between superficial BCC and non-superficial BCC patients were found.

Conclusion We conclude that patients with a superficial BCC differ from non-superficial BCC patients with respect to environmental factors (tumour localization as a proxy for UVR exposure) and phenotypic characteristics (age and sex), but we found no difference in genotype. As superficial BCC patients develop their first BCCs at a younger age, they could be at higher lifetime risk for subsequent skin cancers and therefore be an important group for secondary prevention.

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Conflicts of interest

The authors state no conflict of interest.

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Introduction

Patients with basal cell carcinoma (BCC) put a strain on healthcare services in countries with mainly white-skinned inhabitants, as a result of the high and increasing BCC incidence, and the increased risk of synchronous and metachronous BCCs and other ultraviolet radiation (UVR)-related skin cancers (i.e. field cancerization).[1–3] In addition, the disability-adjusted life years and healthcare costs for BCC have risen significantly as well.[4,5]

There are different histopathological subtypes of BCC, based on the growth pattern(s) found within the tumour tissue. The nodular pattern is the most frequently found histological subtype (>50%), followed by superficial (~20%) and infiltrative (~10%), and about 20% of the tumours show a mixed type.[6–11]

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The frequencies reported depend on the used pathological classification system and period of the study, because classification systems and subtype incidences changed over time.[12,13] BCCs mostly occur on the head and neck area (i.e. chronically sun exposed; >70%), followed by the trunk (~20%) and extremities (~10%), which are both areas intermittently exposed to UVR.[7–10,14] Several observational studies have identified associations between age, sex and anatomical site, and BCC subtypes.[6–9,13] Patients with a superficial BCC more often have their BCC on the trunk and extremities than in the head and neck region,[6–9,13] are younger[7–9,13] and more often female.[8,9] In addition, patients with an initial truncal superficial BCC developed metachronous BCCs at a faster rate than patients with other anatomical site and histology combinations.[15]

These results could indicate that different BCC subtypes, in particular superficial, have other aetiologies with respect to environmental factors (e.g. UVR exposure), phenotypic characteristics (e.g. age and sex) and genetic predisposition. However, only a few studies have studied other predictors than age, sex and anatomical site, with conflicting results.[10,16,17]

The objective of this study was to test the reproducibility of these findings and to find potentially new predictors for patients with a superficial first BCC (sBCC). We hereto analysed the data of almost 1000 white-skinned participants with a BCC of a prospective population-based cohort study (Rotterdam Study).

Materials and methods

Study population

The Rotterdam Study is a prospective population-based cohort study of 14 926 participants (divided over three cohorts) aged 45 years or older, living in a well-defined suburb of Rotterdam, the Netherlands.[18] The cohorts predominantly consist of people of northwestern European descent. All the participants were interviewed and examined at baseline, and these examinations were repeated about every 4 years. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study) and it was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Phenotype/Case definition

The method by which we identified BCCs has been described in detail previously.[19] In short, the study database was linked to the Dutch nationwide network and registry of histopathology and cytopathology (PALGA) to retrieve medical history of all participants on histopathologically confirmed BCCs between 1 July 1989 and 31 December 2013.[20] Of the 14 926 Rotterdam Study participants, 298 did not sign informed consent for a linkage and could not be linked to PALGA. The pathology excerpts we received contained information on date of diagnosis, anatomical location, body side, type of procedure (i.e. biopsy or excision), radicality and diagnosis. The majority of these excerpts showed a subtyping of the BCC, and these subtypes were coded based on the World Health Organization's histological classification of keratinocytic skin tumours.[21] If there was a subtype discrepancy between a biopsy and an excision or a biopsy/excision included more than one subtype, we coded it as a mixed type BCC and noted the concerned subtypes. Patients with a missing subtype were excluded and patients with a mixed type first BCC with a superficial component were excluded as well, because it was unclear to which subtype these belong (i.e. superficial or non-superficial). Metachronous BCCs that occurred within 6 months of the first BCC were counted as additional tumours at the date of the initial diagnosis, as those BCCs were most likely present at this earlier date. We randomly selected a BCC for participants with synchronous BCCs on their first diagnosis date.

Selection of non-genetic candidate predictors

A literature search up to May 2016 for English publications on phenotypic, environmental and tumour-specific factors previously involved in BCC subtypes was done in PubMed. Four phenotypic factors were included, namely age at first BCC, sex, tendency to develop sunburn and pigment status.[7–10,13] The latter was a combination of eye colour and hair colour when young (e.g. a participant with blue eyes and red hair was scored as light). Five environmental characteristics were chosen and concerned a history of being outdoor for over 4 h per day during more than 25 years, sun protective behaviour measured by wearing sunglasses or a hat, smoking, alcohol consumption and coffee consumption.[10,22–24] Finally, two tumour-related variables were included, namely localization of the first BCC and the number of BCCs at first date of diagnosis.[6–9,13,15] All selected variables (except tumour-specific characteristics) were measured at study entry or at a study visit closest to study entry.

Selection of candidate single nucleotide polymorphisms

A literature search up to May 2016 for English genome-wide association study (GWAS) publications of loci that confer risk of BCC or non-melanoma skin cancer was done in PubMed. There was no GWAS of the histopathological subtypes of BCC. To reduce the burden of multiple testing, all selected single nucleotide polymorphisms (SNPs) had to be at least borderline genome-wide significant (P-value $<7.0 \times 10^{-8}$) and had to be replicated in another cohort. This resulted in a list of 20 candidate SNPs located in 17 different chromosomal regions (Table S1).[25]

Genotype

DNA was isolated from whole blood, further processed and quality checked following standard protocols.[18] The Illumina

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Infinium II HumanHap550 BeadChips and the Illumina Human610-Quad BeadChips were used to genotype the Rotterdam Study participants.

Quality control criteria included removing SNPs with Hardy–Weinberg equilibrium deviations (P-value <0.0001), genotyping call rate <97%, gender mismatch and a high mean autosomal heterozygosity. SNPs were not included if they had a minor allele frequency of <1% and/or an imputation r^2 of <0.3.

For the candidate SNP approach, we used genotypes that were estimated from the imputed 1000 Genomes, GIANT Phase I version 3 dosage data[18] using the Genome-wide Complex Trait Analysis (GCTA) software with default parameters.[26] All selected candidate SNPs were included in our genetic database.

Statistical analysis

Non-genetic binary logistic regression analysis of sBCC vs. non-superficial BCC (nsBCC) All the assumptions of a binary logistic regression analysis were tested and we found no violations. There existed no strong (multi)collinearity between the selected non-genetic candidate predictors. A few outliers in the coffee consumption and alcohol consumption variables were found using the outlier labelling rule,[27] but all values were realistic. There was sufficient power to include the 11 selected candidate predictors in the multivariable binary logistic regression analysis.

We could safely assume that missing predictor values were missing at random (i.e. missing data points were not related to the missing data itself, but to the observed data). Missing predictor values could therefore be imputed using multiple imputations (30 times) by an iterative Markov Chain Monte Carlo method. The imputation model included all candidate predictors, the outcome, the body mass index (kg/m^2), the level of education, the side of the first BCC and the Rotterdam Study cohort number. After the imputations, we did both univariable and multivariable binary logistic regression analyses. No selection methods were used for the multivariable analysis.

All of the data management and the non-genetic binary logistic regression analyses were done in IBM® SPSS® Statistics for Windows version 21 (Chicago, IL, USA).

Genetic (SNP-based) binary logistic regression analysis of sBCC vs. nsBCC All the assumptions of a binary logistic regression analysis were tested and we found one violation, namely collinearity between two selected candidate SNPs. A bivariate correlation matrix showed a Pearson correlation coefficient of 0.86 between rs12210050 and rs12202284, which means that these predictors were highly correlated. A few outliers in the age and principal component variables were found using the outlier labelling rule,[27] but all values were realistic. There was insufficient power to include the 20 selected candidate SNPs, age, sex and four principal components (PCs) in the multivariable binary logistic regression analysis. Therefore, we adjusted our analyses for multiple testing using the false discovery rate (FDR).[28] PCs were included to adjust for possible population stratification.

The SNP-based association analyses were performed on the imputed dosage data using a binary logistic regression with an additive model. The multivariable logistic regression analysis was adjusted for age at BCC diagnosis, sex and four PCs. No selection methods were used for the multivariable analysis.

The genetic data were prepared on our genetic servers, and IBM® SPSS® Statistics for Windows version 21 was used for the analyses.

Sensitivity analyses of sBCC vs. nodular BCC We performed sensitivity analyses by doing the same non-genetic and genetic regression analyses as for sBCC vs. nsBCC, but now including only patients with superficial or nodular first BCC.

Results

Study population for non-genetic analyses

Of the 14 628 Rotterdam Study participants linked to PALGA, 1528 had at least one BCC. After the exclusion of patients with a missing subtype (n = 71), patients with a mixed superficial first BCC (n = 58) and patients who developed at least one BCC before study entry (n = 451), 948 eligible BCC patients remained for further analyses. We randomly selected a BCC for participants with synchronous BCCs on their first diagnosis date (n = 125). Of the included patients, 137 (14%) had a superficial first BCC, 496 (52%) a nodular first BCC and the remaining 315 (33%) another subtype (infiltrative, micronodular or non-superficial mixed type; Tables 1 and S2).

Patients with a superficial first BCC were younger than patients with a non-superficial first BCC (median age 70.2 vs. 75.5 years), and the proportion females (64%) were higher in sBCC patients than in nsBCC patients (54%; Table 1). Approximately 4 out of 5 sBCCs were located on the extremities (39%) or trunk (42%) as opposed to 1 in 4 of the nsBCCs.

Non-genetic binary logistic regression analyses of sBCC vs. nsBCC

Of the 11 candidate predictors, 3 were significantly associated with a superficial first BCC in the univariable binary logistic regression analyses, namely a younger age at first BCC diagnosis (OR: 0.94, 95% CI: 0.92–0.96 per year), female gender (OR: 1.47, 95% CI: 1.01–2.14) and localization on the trunk (OR: 11.44, 95% CI: 6.85–19.10) or extremities (OR: 18.07, 95% CI: 10.56–30.93; Table 2).

These associations remained strongly significant after the multivariable binary logistic regression analysis and no other predictors became significant (Table 2). Female gender gave an even stronger risk increase for sBCC (OR: 1.88, 95% CI: 1.16–3.03, P-value <0.05), but localization

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Table 1 Non-genetic characteristics of 948 Rotterdam Study patients with a first BCC

Patient and tumour characteristics	Coding	Overall†	Superficial BCC	Non-superficial BCC
Number of patients		948 (100%)	137 (100%)	811 (100%)
Age at first BCC (years)	Median (IQR)	74.6 (67.9–81.2)	70.2 (64.3–76.0)	75.5 (68.9–81.8)
Sex	Female	526 (55%)	87 (64%)	439 (54%)
Pigment status	Dark	153 (16%)	24 (18%)	129 (16%)
	Intermediate	447 (47%)	70 (51%)	377 (46%)
	Light	213 (22%)	30 (22%)	183 (23%)
	Missing	135 (14%)	13 (9%)	122 (15%)
Easily sunburned	Yes	319 (34%)	53 (39%)	266 (33%)
	Missing	65 (7%)	5 (4%)	60 (7%)
Outdoor work	Yes	124 (13%)	14 (10%)	110 (14%)
	Missing	274 (29%)	42 (31%)	232 (29%)
Sun protection	No, never or hardly ever	357 (38%)	44 (32%)	313 (39%)
	Missing	60 (6%)	4 (3%)	56 (7%)
Smoking	Current or former	623 (66%)	92 (67%)	531 (65%)
	Missing	17 (2%)	1 (1%)	16 (2%)
Alcohol consumption (glasses/day)	Median (IQR)	0.6 (0.1–1.7)	0.6 (0.1–1.4)	0.6 (0.1–1.8)
	Missing	215 (23%)	18 (13%)	197 (24%)
Coffee consumption (cups/day)	Median (IQR)	3.3 (2.0–4.5)	3.3 (1.5–4.0)	4.0 (2.0–5.0)
	Missing	215 (23%)	18 (13%)	197 (24%)
>1 BCC at initial diagnosis	Yes	125 (13%)	24 (18%)	101 (12%)
Localization of first BCC	Head and neck	630 (66%)	24 (18%)	606 (75%)
	Extremities	128 (14%)	54 (39%)	74 (9%)
	Trunk	184 (19%)	58 (42%)	126 (16%)
	Missing	6 (1%)	1 (1%)	5 (1%)

†Participants with a mixed type BCC with a superficial component were excluded.
BCC, basal cell carcinoma; IQR, interquartile range.

remained the strongest predictor (truncal OR: 12.20, 95% CI: 7.08–21.03, *P*-value <0.001; extremities OR: 17.57, 95% CI: 10.06–30.70, *P*-value <0.001). The 11 predictors together explained 19.7% (Cox and Snell R^2) of total variability of a superficial first BCC compared to a non-superficial first BCC.

Study population for genetic analyses

Of the 14 628 Rotterdam Study participants linked to PALGA, 1257 were genotyped and had at least one BCC. After the exclusion of patients with a missing subtype ($n = 181$) and patients with a mixed superficial first BCC ($n = 62$), 1014 eligible BCC patients remained for further analyses. We randomly selected a BCC for participants with synchronous BCCs on their first diagnosis date ($n = 126$). Of the included patients, 159 (16%) had a superficial first BCC, 522 (51%) a nodular first BCC and the remaining 333 (33%) another subtype (infiltrative, micronodular or non-superficial mixed type; Tables 3 and S3).

Patients with a superficial first BCC were younger than patients with a non-superficial first BCC (median age 68.0 vs. 73.5 years), and the proportion females (65%) were higher in sBCC patients than in nsBCC patients (53%).

Genetic (SNP-based) binary logistic regression analyses of sBCC vs. nsBCC

Of the 20 candidate SNPs, 2 were borderline significantly associated with a first sBCC in the univariable SNP-based binary logistic regression analyses, namely rs8015138 (OR: 0.76, 95% CI: 0.60–0.97) and rs12203592 (OR: 1.55, 95% CI: 1.01–2.37; Table 4).

Before the multivariable SNP-based binary logistic regression analyses, we excluded rs12210050 because it was highly correlated (Pearson's *r*: 0.86) with rs12202284 and both SNPs were also in strong linkage disequilibrium (r^2: 0.73) with each other. The multivariable analysis resulted in 1 promising SNP, namely rs12203592 (OR: 1.83, 95% CI: 1.13–2.97, *P*-value 0.014) mapped to pigmentation gene IRF4, but after adjustment for multiple testing (FDR), this SNP lost its significance as well. No other SNPs were significantly associated with sBCC (Table 4).

The 19 candidate SNPs together explained 1.6%, of which rs12203592 explained 0.4% (Cox and Snell R^2), of the total variability of a superficial first BCC compared to a non-superficial first BCC.

Sensitivity analyses of sBCC vs. nodular BCC

After the non-genetic multivariable binary logistic regression analysis comparing sBCC to nodular BCC, the same predictors

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Table 2 Associations between non-genetic predictors and occurrence of superficial first BCC (n = 948)†

Patient and tumour characteristics	Coding	Univariable models‡	Multivariable model‡§
Age at first BCC (years)	Continuous	0.94 (0.92–0.96)***	0.95 (0.93–0.98)***
Sex	Female	1.47 (1.01–2.14)*	1.88 (1.16–3.03)*
Pigment status	Dark	Reference	Reference
	Intermediate	1.01 (0.61–1.67)	0.91 (0.50–1.64)
	Light	0.92 (0.51–1.65)	0.80 (0.40–1.61)
Easily sunburned	Yes	1.22 (0.83–1.78)	1.13 (0.70–1.81)
Outdoor work	Yes	0.77 (0.42–1.38)	0.85 (0.43–1.69)
Sun protection	No or hardly ever	0.70 (0.48–1.04)	0.80 (0.51–1.26)
Smoking	Current or former	1.03 (0.70–1.52)	1.41 (0.85–2.33)
Alcohol consumption (glasses/day)	Continuous	0.90 (0.77–1.05)	0.84 (0.70–1.01)
Coffee consumption (cups/day)	Continuous	0.92 (0.82–1.02)	0.90 (0.79–1.02)
>1 BCC at initial diagnosis	Yes	1.49 (0.92–2.43)	1.41 (0.79–2.52)
Localization of first BCC	Head and neck	Reference	Reference
	Extremities	18.07 (10.56–30.93)***	17.57 (10.06–30.70)***
	Trunk	11.44 (6.85–19.10)***	12.20 (7.08–21.03)***

*P-value <0.05; ***P-value <0.001.
†Compared to nodular, micronodular, infiltrative and mixed type BCCs; all mixed type BCCs with a superficial component were excluded.
‡Pooled ORs with 95% CIs between parentheses.
§Full model, no selection procedures used.
BCC, basal cell carcinoma.

Table 3 Genetic characteristics of 1014 Rotterdam Study patients with a first BCC

Patient and tumour characteristics	Coding	Overall†	Superficial BCC	Non-superficial BCC
Number of patients		1014 (100%)	159 (100%)	855 (100%)
Age at first BCC (years)	Median (IQR)	72.9 (64.4–79.8)	68.0 (60.8–75.6)	73.5 (65.5–80.5)
Sex	Female	556 (55%)	103 (65%)	453 (53%)

†Participants with a mixed type BCC with a superficial component were excluded.
BCC, basal cell carcinoma; IQR, interquartile range.

(age at first BCC diagnosis, sex and a localization on the trunk or extremities) were significantly associated with a superficial first BCC with similar effect sizes (Table S4). The explained variability increased by 4.3% to 25.0% (Cox and Snell R^2).

The multivariable SNP-based binary logistic regression analysis comparing sBCC to nodular BCC resulted in 2 promising SNPs, namely rs12203592 (OR: 2.11, 95% CI: 1.25–3.58, P-value 0.005) and rs12202284 (OR: 0.55, 95% CI: 0.35–0.88, P-value 0.012), both mapped to the IRF4 – EXOC2 region, but were not in strong linkage disequilibrium (r^2: 0.18) with each other (Table S5). However, after adjustment for multiple testing (FDR) both SNPs lost their significance.

Discussion

This prospective population-based cohort study replicates some previous non-genetic findings and shows that there are significant differences between patients with a superficial first BCC and a non-superficial first BCC. Patients who presented with a sBCC were younger, more often female and had their BCCs more frequently on the extremities and trunk than patients with nsBCCs. This study also looked into potential genetic differences. One SNP, mapped to IRF4, looked promising, but after adjustment for multiple testing, no significant differences in genetic make-up between sBCC and nsBCC patients were found.

The associations found between the non-genetic predictors and the occurrence of a superficial first BCC were in line with several other older and more recent observational studies from Europe and Australia.[6–10,13] However, most of these non-genetic studies on histopathological BCC subtypes did not adjust for potential confounders.[6–9,13] Therefore, it is possible that the associations found were spurious. We included 11 potential confounders in our non-genetic multivariable model and found that patients with a superficial first BCC were significantly younger, almost twice as likely to be female and 12–18 times more likely to have their BCC on the trunk or extremities than patients with a non-superficial first BCC. These differences in age, sex and localization could suggest that a different pattern of UVR exposure, namely intense intermittent, plays a role in the aetiology of sBCC as compared to nsBCC. A British and Australian cohort study showed that excessive recreational UVR exposure significantly increased the risk of truncal (superficial) BCCs,[17,29]

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Table 4 Associations between genetic predictors and occurrence of superficial first BCC (n = 1014)†

Patient and tumour characteristics	Coding	Univariable models‡	Multivariable models‡,§	Multivariable model‡,¶
Age at first BCC (years)	Continuous	0.97 (0.95–0.98)***		0.96 (0.95–0.98)***
Sex	Female	1.63 (1.15–2.32)**		1.67 (1.16–2.40)**
rs73635312	Yes	1.11 (0.74–1.67)	1.08 (0.71–1.64)	1.07 (0.70–1.63)
rs11170164	Yes	0.88 (0.57–1.35)	0.93 (0.59–1.45)	0.95 (0.61–1.49)
rs7335046	Yes	0.95 (0.67–1.36)	0.92 (0.64–1.32)	0.89 (0.61–1.29)
rs8015138	Yes	0.76 (0.60–0.97)*	0.78 (0.61–1.00)	0.79 (0.62–1.02)
rs1805007	Yes	0.96 (0.60–1.55)	0.96 (0.59–1.57)	0.95 (0.58–1.56)
rs78378222	Yes	0.89 (0.38–2.10)	0.95 (0.40–2.52)	0.98 (0.41–2.36)
rs7538876	Yes	1.06 (0.82–1.36)	1.02 (0.79–1.32)	1.02 (0.78–1.32)
rs801114	Yes	0.96 (0.75–1.23)	1.03 (0.80–1.33)	1.00 (0.77–1.30)
rs214782	Yes	0.89 (0.66–1.19)	0.91 (0.68–1.22)	0.93 (0.69–1.26)
rs13014235	Yes	1.07 (0.84–1.36)	1.07 (0.83–1.37)	1.07 (0.83–1.38)
rs57244888	Yes	1.06 (0.67–1.67)	1.11 (0.70–1.78)	1.12 (0.70–1.80)
rs401681	Yes	1.05 (0.82–1.33)	1.00 (0.78–1.28)	0.98 (0.76–1.26)
rs12203592	Yes	1.55 (1.01–2.37)*	1.55 (1.01–2.39)*	1.83 (1.13–2.97)*
rs12202284	Yes	0.92 (0.64–1.32)	0.92 (0.63–1.33)	0.72 (0.47–1.08)
rs12210050	Yes	1.00 (0.71–1.40)	1.03 (0.73–1.46)	
rs157935	Yes	0.95 (0.73–1.24)	0.94 (0.72–1.23)	0.95 (0.72–1.26)
rs28727938	Yes	0.89 (0.52–1.53)	0.78 (0.45–1.34)	0.75 (0.43–1.32)
rs7006527	Yes	0.81 (0.58–1.13)	0.79 (0.56–1.12)	0.79 (0.55–1.12)
rs2151280	Yes	1.09 (0.86–1.38)	1.13 (0.89–1.44)	1.14 (0.89–1.46)
rs59586681	Yes	1.09 (0.85–1.40)	1.13 (0.88–1.46)	1.10 (0.85–1.44)

*P-value <0.05; **P-value <0.01; ***P-value <0.001.
†Compared to nodular, micronodular, infiltrative and mixed type BCCs; all mixed type BCCs with a superficial component were excluded.
‡Odds ratios with 95% confidence intervals between parentheses.
§Included one SNP at a time, adjusted for age at first BCC, sex and first 4 principal components.
¶Full model, adjusted for age at first BCC, sex and first 4 principal components. No selection procedures used.
BCC, basal cell carcinoma.

whereas Dutch and Italian case–control studies showed no relation between cumulative lifetime UVR exposure and sBCC.[10,16]

Another potential explanation for the significantly higher risk of sBCC in younger women could be behaviour. Women tend to use tanning beds more often than men[30,31] and pay closer attention to their health and physical appearance than men, which may lead to more medical visits.[32]

It is also possible that tumour biology differs at various anatomical sites. A Dutch renal transplant study showed that transplant recipients more often developed sBCCs and that their BCCs were located more frequently on the trunk and extremities than in the non-immunosuppressed, which may point at role for the immune system.[8]

Superficial first BCC patients were significantly younger (approximately 5 years) than non-superficial first BCC patients and developed their BCCs more often on relatively sun-unexposed sites, which could mean that they have a different genetic predisposition which makes them more vulnerable to develop (superficial) BCC. It is possible that they, for example, have a reduced DNA repair capacity or other risk-increasing DNA differences.[33] Hence, we compared carefully selected BCC candidate SNPs between these two patient groups. Of the 19 included candidate SNPs in the multivariable regression analysis, rs12203592 looked most promising (OR: 1.83, 95% CI: 1.13–2.97, P-value 0.014), but lost its significance after adjusting the FDR. This SNP is an intron variant mapped to the interferon regulatory factor 4 (IRF4) gene, which belongs to a well-known family of transcription factors that are important in the regulation of the immune system. It is possible that certain SNPs downregulate the immune system which could lead to the formation of sBCC in relatively sun-unexposed areas earlier in life. A recent genetic analysis of melanoma patients showed a significant association with the bimodal (early- and late-onset) age distribution of melanoma for different rs12203592 genotypes.[34] In addition, IRF4 also plays a key role in the pigmentation pathway and in the formation of (pre)malignancies of the skin.[35–37] These premalignancies (i.e. actinic keratosis) have a superficial growth pattern which is comparable to that of sBCCs.

Limitations

Misclassification of BCC subtypes by pathologists most likely occurred throughout the study period, but it is unlikely that this misclassification was differential. However, we could not check the tissue samples as we only received excerpts from PALGA.

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The total number of BCCs could have been underestimated, since we only included histopathologically confirmed BCCs. This underestimation will be most pronounced for superficial BCCs, because physicians could diagnose these BCCs visually and treat them non-invasively. However, a recent Dutch observational study showed that only a small percentage (ca. 7%) of patients with metachronous BCCs had subsequent non-histologically confirmed BCCs.[38] In addition, the evidence-based BCC guideline from the Dutch Society for Dermatology and Venereology states that histopathological verification is needed for all for BCC suspicious lesions.[39] Finally, the distribution pattern of the histopathological subtypes in our study population is in line with other studies, with the nodular type being the most common, followed by the superficial type and infiltrative type, while mixed types were frequently found as well.[6–11] Our candidate SNP approach likely lacked sufficient power (26 degrees of freedom used and 159 patients with a superficial first BCC) despite the FDR approach taken. Detailed information about other limitations of the Rotterdam Study, the phenotype collection and the non-genetic and genetic predictors can be found in two earlier publications.[19,40]

Conclusion

Patients with a superficial first BCC differ from non-superficial first BCC patients with respect to environmental factors (tumour localization as a proxy for UVR exposure) and phenotypic characteristics (age and sex), but (as far as we could find) not in genotype. As sBCC patients develop their first BCCs at a younger age, they could be at higher risk for subsequent skin cancers. Further study of the interplay between environmental, phenotypic and genotypic predictors and BCC subtypes may provide useful knowledge for BCC pathogenesis and the design of programs for prevention and early detection of BCC.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Candidate single nucleotide polymorphisms for basal cell carcinoma or non-melanoma skin cancer.
Table S2. Non-genetic characteristics of 633 Rotterdam Study patients with a primary BCC.
Table S3. Genetic characteristics of 681 Rotterdam Study patients with a primary BCC.
Table S4. Associations between non-genetic predictors and occurrence of superficial first BCC (n = 633).
Table S5. Associations between genetic predictors and occurrence of superficial first BCC (n = 681).

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