BJD
British Journal of Dermatology

Iris pigmented lesions as a marker of cutaneous melanoma risk: an Australian case–control study*

A.M. Laino,[1] E.G. Berry,[1,2] K. Jagirdar,[1] K.J. Lee,[1] D.L. Duffy,[1,3] H.P. Soyer[1,4] and R.A. Sturm[1]

[1]Dermatology Research Centre, The University of Queensland, UQ Diamantina Institute, Translational Research Institute, Brisbane 4102, Australia
[2]Department of Dermatology, Emory University School of Medicine, Atlanta 30309, GA, U.S.A.
[3]QIMR Berghofer Medical Research Institute, Brisbane 4006, Australia
[4]Department of Dermatology, Princess Alexandra Hospital, Brisbane 4102, Australia

Summary

Background Iris naevi and iris freckles have a frequency of 4% and 50% in the European population, respectively. They are associated with dysplastic naevi, but few studies have examined their link to cutaneous melanoma.
Objectives To assess whether iris pigmented lesions are a predictive indicator for cutaneous melanoma.
Methods This is a melanoma case–control study of 1254 European-background Australians. Sun exposure and melanoma history, a saliva sample for DNA analysis and eye photographs taken with a digital camera were collected from 1117 participants. Iris images were assessed by up to four trained observers for the number of iris pigmented lesions. The data were analysed for correlations between iris pigmented lesions and melanoma history.
Results Case participants over the age of 40 had similar numbers of iris pigmented lesions to age matched controls (mean 5·7 vs. 5·2, $P = 0·02$), but in younger case and control participants there was a greater difference (mean 3·96 vs. 2·19, $P = 0·004$). A logistic regression adjusted for age, sex, skin, hair and eye colour, skin freckling and naevus count found that the presence of three or more iris pigmented lesions increases the melanoma risk 1·45-fold [95% confidence interval (CI) 1·07–1·95]. *HERC2/OCA2* rs12913832 and *IRF4* rs12203592 influenced both eye colour and the number of iris pigmented lesions. On the *HERC2/OCA2* A/A and A/G genotype background there was an increasing proportion of blue eye colour when carrying the *IRF4* T allele ($P = 3 \times 10^{-4}$) and a higher number of iris pigmented lesions with the *IRF4* T/T homozygote ($P = 3 \times 10^{-9}$).
Conclusions Iris pigmented lesion count provides additional predictive information for melanoma risk above that from conventional risk factors.

Correspondence
Richard A. Sturm.
E-mail: r.sturm@uq.edu.au

Accepted for publication
27 December 2017

Funding sources
This work was supported by the Australian National Health and Medical Research Council (NHMRC project grants APP1062935, APP1083612 and the Centre of Research Excellence for the Study of Naevi APP1099021). R.A.S. is a senior research fellow of the Australian NHMRC. The funder had no role in the study design, data collection, data analysis, manuscript preparation and/or publication decisions.

Conflicts of interest
None to declare.

*Plain language summary available online

DOI 10.1111/bjd.16323

What's already known about this topic?

- Clinicians should be aware of groups at high risk for melanoma to facilitate early detection; melanoma survival rates remain highest when the melanoma is identified at a localized stage and drop steeply if the melanoma has spread regionally.
- Well-established melanoma risk factors include phenotypic characteristics such as skin type and numbers of melanocytic naevi on the skin.

What does this study add?

- We found a 45% increased risk of cutaneous melanoma associated with the presence of three or more iris pigmented lesions.
- This association was particularly strong in study participants aged 40 years or under.

- Iris pigmented lesion count therefore provides additional predictive information for melanoma risk stratification over and above currently used phenotypic factors such as skin type, hair and eye colour and naevus count.

The number of melanocytic naevi and dysplastic naevi are well-known risk factors for melanoma.[1] However, less is known about the relationship between iris naevi, iris freckles and cutaneous melanoma. Iris pigmented lesions are common in the European population, with a frequency of 2–6% for iris naevi and 25–60% for iris freckles.[2,3] Iris naevi are benign melanocytic proliferations of the iris; they are raised, well-circumscribed brown lesions which form a nodule that replaces the iris stroma.[2,4,5] So-called iris freckles are also clusters of hyperpigmented melanocytes, unlike adult cutaneous freckles that are areas with accumulated melanin levels but no extra melanocytes.[6] Iris freckles present as flat, pigmented lesions on the anterior iris surface that do not disrupt the normal architecture of the iris.[2,5,7] As these traits appear at high frequency, elucidating the association of the number of iris pigmented lesions and cutaneous melanoma could serve as an additional tool for melanoma risk stratification.

A connection between ocular and cutaneous melanocyte behaviour can be expected, given their common embryological origin from neural crest cells.[2,3] Several studies have reported higher numbers of iris pigmented lesions in patients with dysplastic or atypical naevi.[8,9] Other groups have reported a positive correlation between dysplastic naevus syndrome and uveal melanoma.[10,11] A history of painful sunburns during childhood and a lifestyle involving sun exposure have been associated with increased numbers of melanocytic naevi, higher degree of skin freckling and increased melanoma risk.[6,12] However, data on ultraviolet (UV) exposure as a risk factor for the development of ocular melanoma and iris naevi is equivocal.[7,11,13] Schwab *et al.* have shown that a history of sunburns, use of sunglasses and signs of sun damaged skin had no significant association with total numbers of iris naevi.[7]

Cutaneous melanoma is more common in people with a fair complexion,[1] and genes influencing skin colour, skin freckling and melanocytic naevus formation have been extensively researched. *MC1R*, *OCA2*, *IRF4*, *ASIP*, *TYR* and *BNC2* variants are important for the formation of freckles on the skin[6,14,15] and *MTAP*, *PLA2G6*, *IRF4* and *MITF* variants influence melanocytic naevus count.[16] Genes determining eye colour are also well-investigated, with just one single-nucleotide polymorphism (rs12913832) in intron 86 of *HERC2* immediately upstream of *OCA2* explaining most variation in blue-brown eye colour.[17]

This study evaluated the association between iris pigmented lesions and melanoma, and whether the presence of iris pigmented lesions scored from digital photographic images can be an indicator of cutaneous melanoma risk. We also evaluated whether the genes influencing iris lesions are the same as for cutaneous melanocytic naevi and freckling.

Patients and methods

This study was approved by the Human Research Ethics Committee of Princess Alexandra Hospital and The University of Queensland, and conducted in accordance with the Declaration of Helsinki.

Study population

A total of 1254 individuals volunteered for clinical examination from October 2009 to November 2015 as part of the Brisbane Naevus Morphology Study (BNMS), an epidemiological study of naevus and melanoma genes in participants recruited from South-East Queensland.[18,19] Participants were recruited from the Melanoma Unit and Dermatology Department of the Princess Alexandra Hospital (Brisbane, Australia), private dermatology clinics, the Brisbane Longitudinal Twin Study (BLTS) and the QSkin Study.[20] There was no age limit, but participants who were unable to stand for the duration of the skin imaging were excluded from the study. Case participants had a personal history of one or more primary melanomas, verified by the referring dermatologist or attendance at the Melanoma Unit; unaffected control participants had no personal history of melanoma, as reported by the participant, and were predominantly drawn from the BLTS and QSkin to collect a population phenotypically representative of the European-background population in Queensland. A 60-participant pilot conducted from October 2009 to March 2010 established that a maximum of 100 case and 100 control participants could be imaged per year. The study length was determined by funding constraints.

Data collection

Participants completed a questionnaire regarding sun exposure history, sun sensitivity, use of sunglasses, hats and sunscreen, past medical history and their natural hair colour at the age of 21 years. A research assistant recorded the participant's eye and skin colour, and degree of skin freckling on the face, shoulders and dorsal hands. A count of large naevi ($\geq$ 5 mm) was made from full body images captured with a Vectra® WB360 3D body imager (Canfield Scientific Inc., Parsippany, NJ, U.S.A.) or FotoFinder® (Bad Birnbach, Germany).

Iris imaging

Iris images were captured with a Powershot SD770IS digital camera (Canon, Lake Success, NY, U.S.A.; examples in Fig. 1). Four researchers were trained in identifying iris pigmented

lesions and peripupillary ring (a brown hyperpigmented zone immediately around the pupil)[21,22] with images from a training set of 63 participants. The remaining images were independently examined by two to four researchers, and assessed for iris colour (blue/grey, green/hazel or brown), presence or absence of a peripupillary ring, and total number of iris pigmented lesions. We did not distinguish between iris naevi and freckles as this requires slit-lamp examination of the participants[2,7] and cannot be ascertained from eye images alone.

Biospecimen collection

Genomic DNA was extracted from 2 mL of saliva collected using an Oragene-DNA self-collection kit (DNA Genotek, Ottawa, ON, Canada).[18] A minimum of 2·5 μg of DNA was processed at the UQ Centre for Clinical Genomics on an Illumina Infinium HumanCoreExome-24 Microarray (Illumina, San Diego, CA, U.S.A.).[19]

Statistical analysis

Statistical analysis used the Sib-pair 1.0.0 computer program[23] and the R-package.[24] Assessments of eye colour, peripupillary ring and number of iris pigmented lesions were tested for interobserver reliability by calculating a rank correlation coefficient (Fig. S1; see Supporting Information). Spearman's rank correlation coefficients were calculated for each eye colour class to determine the consistency between iris colour assessment at the participant visit and later from digital images. Linear and binary logistic regressions were used to assess the importance of phenotypic and genotypic risk factors for predicting both iris features and melanoma risk; missing values were excluded during the analysis. Heritability of log iris pigmented lesion count was estimated using a mixed model that included age and melanoma status as covariates.

Results

Phenotypic iris characteristics of Brisbane Naevus Morphology Study participants

Of the 1254 BNMS participants, 1117 had iris photographs of sufficient quality to be assessed for presence of iris pigmented lesions. The demographic and phenotypic characteristics of the 1117 participants are presented in Table 1. Participants were predominantly of European descent and had fair skin with a varying degree of sunburn propensity and tanning response. The mean age of all participants was 47 years, with men slightly older than women. The most common eye colour called from the digital images was blue/grey (66·4%), followed by green/hazel (23·4%) and brown (10·2%). The majority of eyes examined had at least one pigmented lesion (76%).

Iris pigmented lesions were more common in green/hazel eyes (Fig. S2a; see Supporting Information) but did not vary significantly with skin colour after adjusting for iris colour (Fig. S2b). The number of iris pigmented lesions increased with age (Fig. 2). There was a high level of agreement between researchers for total iris pigmented lesion count. The Spearman correlation varied between 0·77 and 0·94 across observer pairs (Fig. S1; see Supporting Information). Differences in iris pigmented lesion counts were most commonly because of poor image quality. There was also greater difficulty in assessing iris pigmented lesions in brown eyes.

There was a high correlation (Spearman r = 0·8) between iris colour assessment at the time of the participant visit and interpretation from digital images; concordance from participant visits vs. digital readings for observer 1 were blue/grey, 0·84; green/hazel, 0·76; and brown, 0·92. Variations in ambient lighting when conducting participant interviews is the most likely cause of the discordance in eye colour calling, with more precise calls being made with digital imaging.

Relationship between iris pigmented lesion count, naevus count, pigmentation phenotype and melanoma

Case participants had fairer skin, hair and eyes and higher melanocytic naevus counts than the unaffected controls (Table 1, and Fig. S3; see Supporting Information). Notably, the presence of a peripupillary ring was not associated with melanoma.

Presence of iris pigmented lesions was associated with cutaneous melanoma (Table 1, Fig. 2, unadjusted Spearman correlation r = 0·2). The mean number of iris pigmented lesions was 5·4 in the case participants and 3·4 in the controls. The difference between case and control participants was greater in people ≤ 40 years (mean 3·96 vs. 2·19, P = 0·004) than between case and control participants aged > 40 years (mean 5·7 vs. 5·2, P = 0·02). Logistic regression adjusting for age, sex, skin, hair and eye colour, freckling and naevus count (≥ 5 mm) found that the presence of three or more iris pigmented lesions increases the melanoma risk 1·45-fold [Table 2, P = 0·02, 95% confidence interval (CI) 1·07–1·95].

Association between iris pigmented lesion count and genes associated with pigmentation and naevi

Iris pigmented lesion counts were highest in green/hazel irides, with a mean of 5·4 pigmented lesions, compared with 4·4 in blue and 1·5 in brown (Fig. S2a shows median distribution; see Supporting Information). There was an underlying genetic association with the two most important genes for eye colour, *HERC2/OCA2* and *IRF4*.[22] *HERC2/OCA2* rs12913832*A/G and *IRF4* rs12203592*T/C affected both eye colour and the number of iris pigmented lesions (Fig. 3, and Fig. S4; see Supporting Information). *HERC2/OCA2* rs12913832 (A allele for brown, G allele for blue) was the primary driver of eye colour, but its effect was modified by *IRF4* rs12203592 (C allele darkens, T allele lightens).

Blue eye colour was strongly associated with the *HERC2/OCA2* rs12913832 G/G genotype, where there was little change in the frequency of blue eye (> 90%) colour regardless of *IRF4* genotype. However, in both *HERC2/OCA2* heterozygote A/G and homozygote A/A genotypes there was

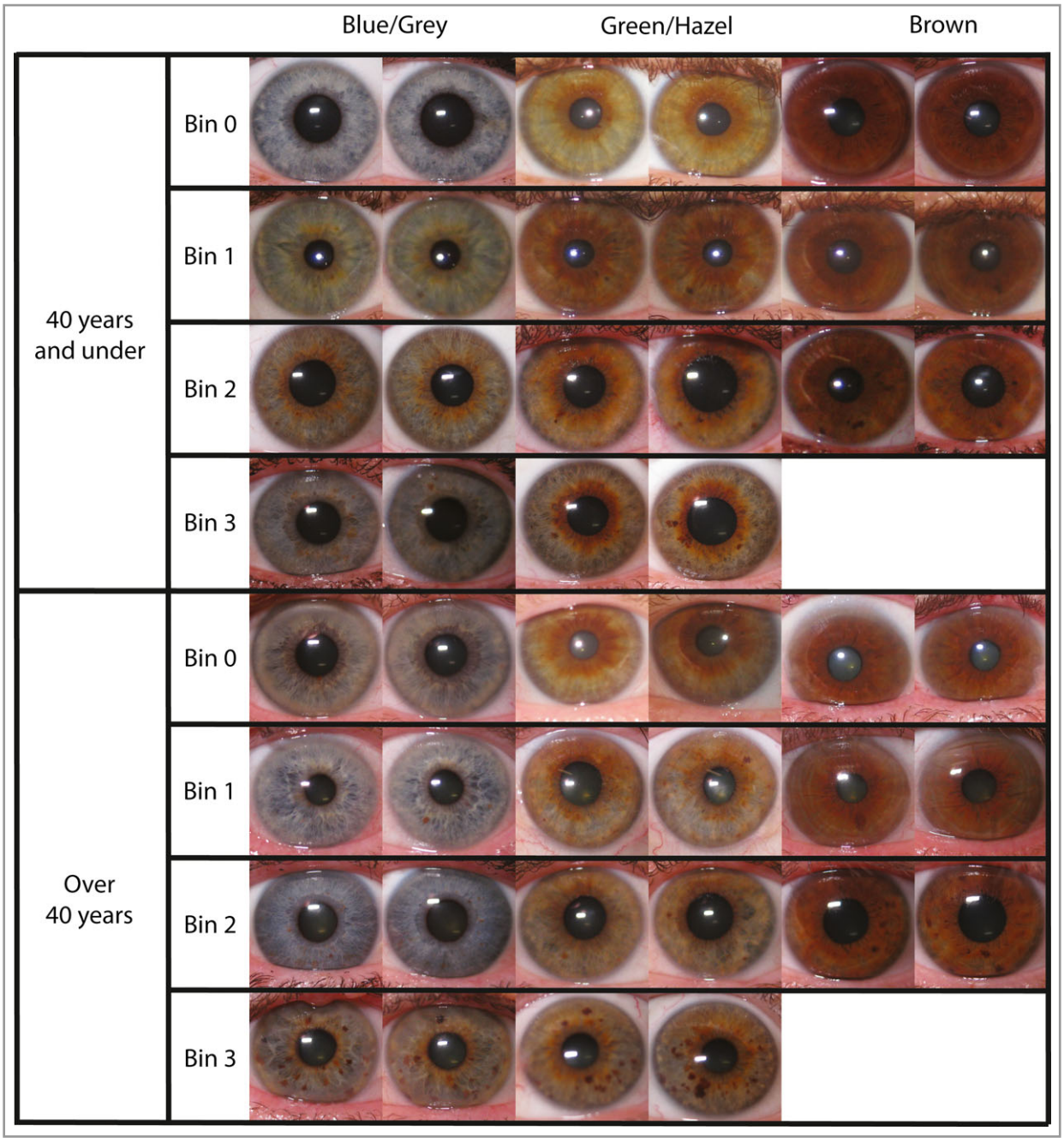

Fig 1. Digital images from blue/grey, green/hazel and brown irides from bins 0 (0 lesions), 1 (1–3 lesions), 2 (4–10 lesions), and 3 (≥ 11 lesions). Upper panel: ≤ 40 years; lower panel: > 40 years.

an increasing proportion of blue eye colour when carrying the *IRF4* rs12203592 T allele, with the *IRF4* T/T homozygote having 64% and 20% blue, respectively, on these genetic backgrounds ($P = 3 \times 10^{-4}$, Fig. S4; see Supporting Information).

This interaction is also reflected in the number of iris pigmented lesions, based on the number of *IRF4* rs12203592*T alleles. The mean count is similar for the *HERC2/OCA2* rs12913832 G/G genotype but the *IRF4* T/T homozygote is associated with higher numbers of iris pigmented lesions in *HERC2/OCA2* rs12913832 A/A or A/G individuals ($P = 3 \times 10^{-9}$; Fig. 3, and Fig. S4; see Supporting Information).

Previous work[17,25,26] examining *OCA2* coding variants found that rs1800401*C/T (Arg305Trp), rs1800407*G/A (p.Arg419Gln), rs74653330*A/T (p.Ala481Thr) and rs121918166*G/A (p.Val443Ile) were important in predicting eye colour variation, and were hypothesized to work as penetrance modifiers. Considering these additional variants, we found that Arg419Gln was the only coding polymorphism to

Table 1 Demographic and phenotypic characteristics of participants

Characteristic	Total	Control participants[a]	Case participants[b]	P-value[c]
Age, years: mean ± SD				
Men	50·0 ± 17·8	40·5 ± 15·4	58·9 ± 15·1	
Women	44·4 ± 15·8	39·1 ± 14·2	51·2 ± 15·1	
Total	47·1 ± 17·0	39·7 ± 14·7	55·2 ± 15·6	
Sex				
Men	542 (48·5)	262 (44·7)	280 (52·7)	0·009
Women	575 (51·5)	324 (55·3)	251 (47·3)	
Total N	1117	586	531	
Skin colour				
Fair	865 (78·0)	445 (75·9)	420 (80·3)	0·02
Medium	209 (18·8)	115 (19·6)	94 (18·0)	
Olive	35 (3·2)	26 (4·4)	9 (1·7)	
Subtotal n	1109	586	523	
Hair colour				
Blonde	211 (19·0)	110 (18·9)	101 (19·1)	< 0·001
Red	123 (11·1)	39 (6·7)	84 (15·9)	
Light brown	372 (33·5)	216 (37·0)	156 (29·5)	
Dark brown	354 (31·9)	191 (32·8)	163 (30·9)	
Black	51 (4·6)	27 (4·6)	24 (4·5)	
Subtotal n	1111	583	528	
Eye colour[d]				
Blue/grey	739 (66·4)	366 (62·7)	373 (70·5)	< 0·001
Green/hazel	261 (23·4)	138 (23·6)	123 (23·2)	
Brown	113 (10·2)	80 (13·7)	33 (6·2)	
Subtotal n	1113	584	529	
Peripupillary ring[d]				
Present	254 (22·8)	136 (23·3)	118 (22·3)	0·75
Absent	859 (77·2)	448 (76·7)	411 (77·7)	
Subtotal n	1113	584	529	
Presence of ≥ 3 iris pigmented lesions in participants > 40 years				
Yes	325 (48·9)	99 (42·1)	226 (52·6)	0·01
No	340 (51·1)	136 (57·9)	204 (47·4)	
Subtotal n	665	235	430	
Presence of ≥ 3 iris pigmented lesions in participants ≤ 40 years				
Yes	119 (26·3)	77 (21·9)	42 (41·6)	< 0·001
No	333 (73·7)	274 (78·1)	59 (58·4)	
Subtotal n	452	351	101	

Values are n (%) unless otherwise indicated. [a]Unaffected participant with no prior history of melanoma; [b]case participant with prior history of at least one melanoma; [c]χ^2-test, unadjusted for other covariates; [d]Observer 1 recorded reads for the 1117 total study participants included for all observers.

interact with *IRF4* T/T genotype in predicting iris pigmented lesion counts, but only on a *HERC2/OCA2* rs12913832 G/A heterozygote background, with a linear trend for mean pigmented lesion counts of 4·5 (419Arg/Arg), 6·5 (419Arg/Gln) and 8·0 (419Gln/Gln).

The peripupillary ring occurred most often in the *HERC2/OCA2* rs12913832 A/G heterozygote (blue iris with a ring 60%, green iris with a ring 82%, Table S1; see Supporting Information) and in green/hazel eyes. The *IRF4* genotype did not influence the presence of the ring (Fig. 3 and Fig. S4)

Iris pigmented lesion count vs. skin freckling

Facial freckling did not differ between cases and controls, but there was more freckling on the shoulders and hands in case participants, as expected. Skin freckling at any site was not associated with iris pigmented lesions when added to the multivariate regression (Table S2; see Supporting Information). Furthermore, self-reported sun exposure showed no relationship with iris pigmented lesions.

Discussion

In this study, we compared the number of iris pigmented lesions in melanoma case participants with that in unaffected controls. We found that the number of iris pigmented lesions was associated with cutaneous melanoma risk even after adjusting for known predisposing host factors such as skin and eye colour, skin freckling and naevus count.

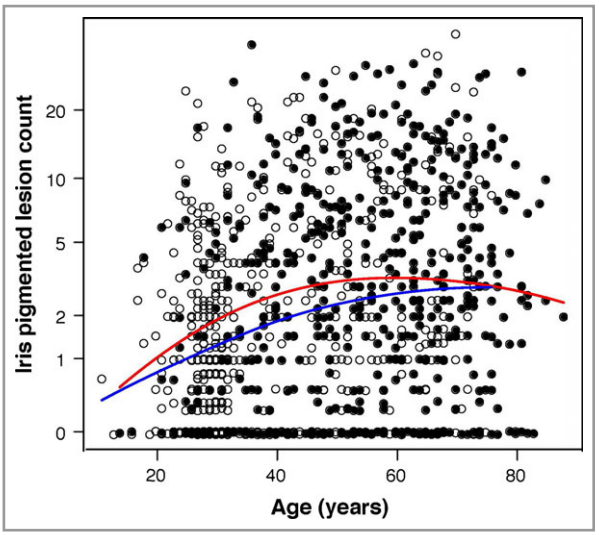

Fig 2. Scatter plot of mean estimated iris pigmented lesion count (four observers) vs. study participant age. Closed circles represent melanoma cases and open circles controls. The localized regression line is red for cases and blue for controls.

Australia and New Zealand have the highest rates of melanoma in the world, and 9·9% of Australians diagnosed with melanoma in 2012 were under the age of 40.[27] The development of new treatments, such as immunotherapy and targeted molecular therapy, has improved overall survival in selected patients; however, long-term prognosis remains poor[28,29] and early detection of melanoma remains critical to reducing disease burden.

The phenotypic features associated with a higher risk of melanoma help to identify patients who may benefit from regular skin examinations to aid early detection. Red or fair hair, skin freckling, fair skin, light eye colour and dysplastic naevi are all features associated with an increased risk of melanoma. In 1985, Nordlund *et al.* found that the total number of iris pigmented lesions was significantly greater in melanoma patients than in controls ($P < 0{\cdot}01$),[30] yet there is a lack of further literature exploring this association.

We detected at least one iris pigmented lesion in 76% of the participants in this study, which is higher than the incidence of 25–60% reported in most studies.[2,3] A recent study from Schwab *et al.*[31] found that 76% of Austrians participating in a skin cancer screening campaign had one or more iris pigmented lesions. That population is comparable with our control group (Austrians with fair skin 75% vs. Australian controls 76%; light coloured eyes 53% vs. 62·7%). The strong association between iris pigmented lesions and dysplastic naevus syndrome reported in earlier studies,[9] and the common origin of cutaneous and ocular melanocytes, suggests that an association between iris pigmented lesions and cutaneous melanoma should be expected. We found that in participants of all ages, the presence of three or more iris pigmented lesions was associated with a 45% increased risk of cutaneous melanoma. This association was particularly obvious for those ≤ 40 years of age with an odds ratio of 1·8 (95% CI 1·03–3·14, Table 2).

Table 2 Logistic regression predicting melanoma status

Model term	Odds ratio	Confidence interval		Likelihood ratio test, P-value
		2·5%	97·5%	
Linear and quadratic age	–	–	–	< 0·001
Female sex	1·03	0·77	1·39	0·06
Skin colour, olive (reference)[a]	1			0·002
Fair	0·96	0·34	2·86	
Medium	0·73	0·25	2·23	
Hair colour, black (reference)[a]	1			0·003
Red	2·21	0·91	5·33	
Fair	1·24	0·54	2·82	
Light brown	1·11	0·50	2·46	
Dark brown	1·24	0·57	2·72	
Iris colour, brown/black (reference)	1			0·10
Blue/grey	1·36	0·79	2·37	
Green/hazel	1·46	0·81	2·64	
Freckling shoulders (4-point scale)	1·33	1·13	1·55	< 0·001
Log_{10} naevus count (≥ 5 mm)	3·93	2·84	5·51	< 0·001
Iris pigmented lesion count ≥ 3	1·45	1·07	1·95	0·02
Subgroup analysis: age, years				0·18[b]
Age ≤ 40, iris pigmented lesion count ≥ 3	1·80	1·03	3·14	
Age > 40, iris pigmented lesion count ≥ 3	1·17	0·81	1·70	

[a]Although the confidence intervals include the value 1, the likelihood ratio test for skin and hair colour is significant. [b]Likelihood ratio test P-value for age heterogeneity of association between iris pigmented lesions and melanoma.

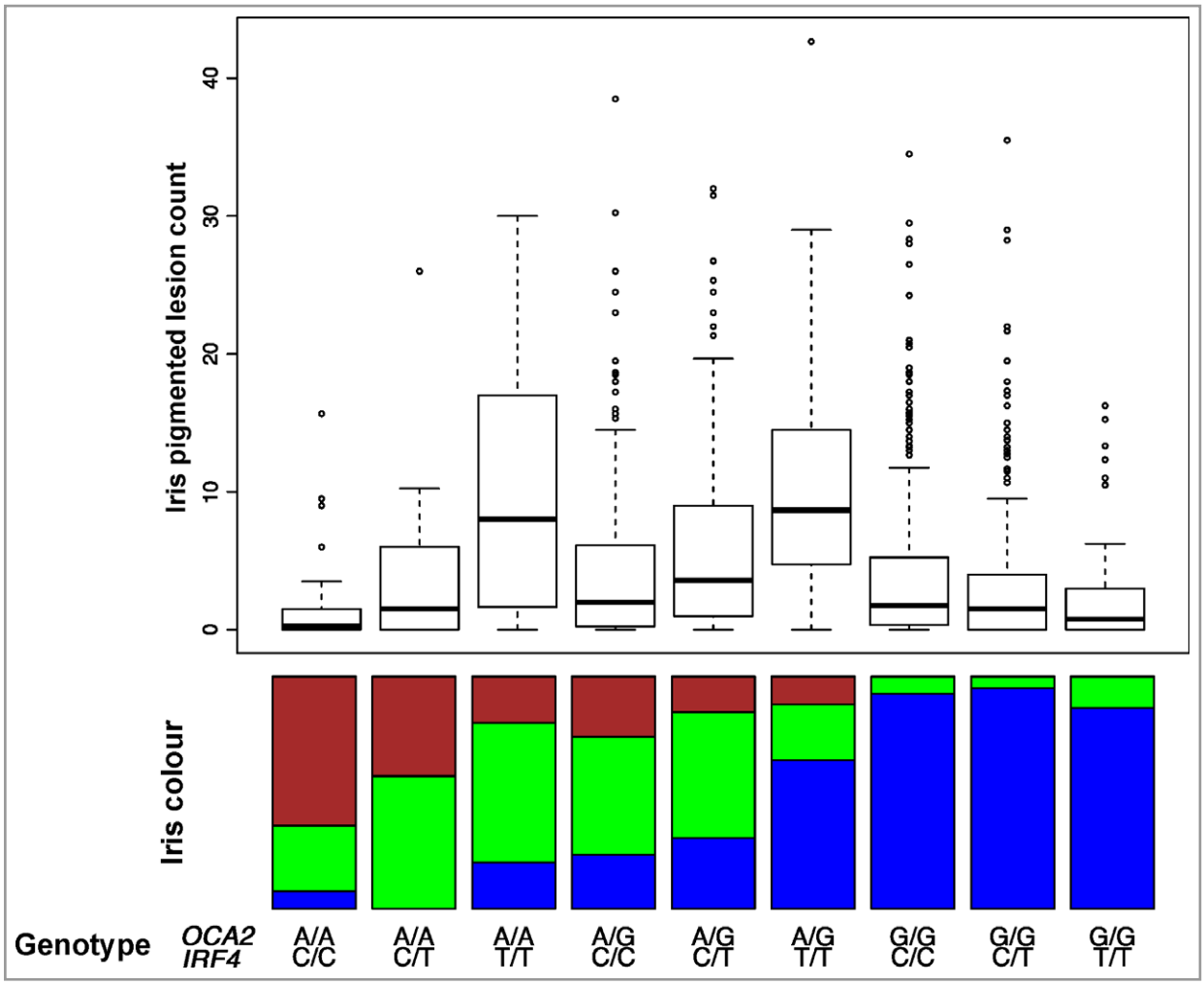

Fig 3. Upper panel: plot of mean iris pigmented lesion count; lower panel: proportion of each digital iris colour, blue for blue/grey, green for green/hazel and brown for brown. *OCA2* rs12913832*A/G and *IRF4* rs12203592*T/C genotypes are indicated on the *x*-axis.

Under the divergent pathway model there are two groups of individuals who develop melanoma: those with a long history of sun exposure, which is generally an older population, and those who have a propensity to develop naevi, in which sun exposure is less critical and melanoma develops at a younger age.[32,33] As iris pigmented lesions are markers for melanoma risk in younger patients, the presence of iris pigmented lesions may identify individuals in the second pathway, who have an inherently high propensity for melanocyte proliferation and transformation into melanoma.[33] This is supported by current literature showing that although some types of ocular melanoma are related to UV radiation, other forms of ocular melanoma and iris pigmented lesions are not clearly related to UV radiation.[7,11,34] It seems likely that the analysis reaching the contrary conclusion by Schwab *et al.*[31] does not adjust for age, an important correlate of iris pigmented lesion count.

In our Australian population there was also no association between skin freckling, sun exposure and the presence of iris pigmented lesions. This suggests again that iris pigmented lesions would be more prevalent in individuals who are 'naevus prone', and exhibit less solar damage, higher numbers of melanocytic naevi and develop melanoma earlier in life.[33] Interestingly, our study found that iris pigmented lesions are more frequent with increasing age (Fig. 2): 48·9% of participants > 40 years of age had three or more pigmented lesions compared with 26·3% of participants ≤ 40 years of age. Csoma *et al.* and Schwab *et al.* also noted greater numbers of iris pigmented lesions with increasing age.[2,31] Although our study found no association between sun exposure and the presence of iris pigmented lesions, the development of iris pigmented lesions over time suggests that perhaps there is a threshold UV level required for expression of iris pigmented lesions.

Melanocytic naevi remain clinically dynamic throughout an individual's life, with increasing numbers as one approaches middle age and regression in the later decades of life.[35] Additionally, total body naevus count has been shown to be a highly heritable trait;[36,37] our results, which include 57 twin pairs, show that iris pigmented lesion count is as heritable (Table S3; see Supporting Information) as body naevus

count,[38] at 72%. This is consistent with our findings that *OCA2* and *IRF4* are major contributors to the phenotype.

Light eye colour is an established risk factor for melanoma.[39] In our study there were higher numbers of iris pigmented lesions in green/hazel eyes. We found no significant difference in the incidence of melanoma between blue/grey and green/hazel eye colour groups in our study population, although melanoma case participants had more iris pigmented lesions across all eye colour classes. In fact, iris pigmented lesions were more strongly associated with melanoma than eye colour (Table 2).

Our study highlighted two genes, *OCA2* and *IRF4*, that were associated with iris colour and iris pigmented lesions. *OCA2* does not have a large effect on skin freckling and naevus count,[40] whereas *IRF4* is a major gene for skin freckling and naevi[41] and has a growth regulatory effect on cutaneous melanocytes.[42] We found that other known melanocytic naevus genes, *MTAP* (rs7023329) and *PLA2G6* (rs132972), are not associated with iris pigmented lesions.[16] Despite their common embryological origin, it appears that the spectrum of genes influencing the development of iris pigmented lesions are different from their cutaneous counterparts (Table S4; see Supporting Information).

We have confirmed previous findings regarding the genes determining eye colour and presence of a peripupillary ring.[43] The *IRF4* rs12203592*T allele has been associated with fair skin, brown hair, and higher total body naevus counts in adolescence, in addition to blue eye colour. The T/T genotype was more common in individuals with blue eyes and C/C in those with brown eyes, which is consistent with results from our study. We also found that individuals with heterozygous *HERC2/OCA2* rs12913832 A/G genotype with blue and green eyes were more likely to have a peripupillary ring than those with brown eyes. However, peripupillary rings may simply be more difficult to distinguish in dark eyes. Major polymorphisms in the *SLC24A4* rs12896399*G/T and *SLC24A5* rs1426654*G/A genes were also found to have a weak effect on peripupillary ring.

There are several potential limitations to this study. Eye image quality was variable, but participants with images that were too blurry to count pigmented lesions were excluded. The good correlation between observers (Fig. S1; see Supportng Information) suggests that images remaining were of sufficient quality to count pigmented lesions reliably. It was also impossible to distinguish iris naevi from iris freckles using digital images; a future prospective study should use slit-lamp examination to distinguish naevi from freckles, and assess whether either category has a greater influence on melanoma risk. Finally, the mean age of control participants was significantly younger than that of case participants (39·7 vs. 55·2 years, respectively); however, this was accounted for in the logistic regression.

Our study shows that the presence of iris pigmented lesions in patients who are 40 years of age or under increases the odds of developing cutaneous melanoma approximately 1·8-fold. Furthermore, the risk rises proportionally to the number of iris pigmented lesions. These findings suggest that a detailed dermatological examination for melanoma may be justified in patients under age 40 noted to have iris pigmented lesions. Future studies are required to define this association further, including identifying the genes influencing iris pigmented lesion formation.

References

1 Gandini S, Sera F, Cattaruzza MS *et al.* Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. *Eur J Cancer* 2005; **41**:28–44.

2 Csoma RZ, Toth-Molnar E, Varga A *et al.* Risk factors and relationship of cutaneous and uveal melanocytic lesions in monozygotic and dizygotic twin pairs. *PLOS ONE* 2016; **11**:e0160146.

3 Harbour JW, Brantley MA Jr, Hollingsworth H, Gordon M. Association between posterior uveal melanoma and iris freckles, iris naevi, and choroidal naevi. *Br J Ophthalmol* 2004; **88**:36–8.

4 Grin JM, Grant-Kels JM, Grin CM *et al.* Ocular melanomas and melanocytic lesions of the eye. *J Am Acad Dermatol* 1998; **38**:716–30.

5 Eagle RC Jr. Iris pigmentation and pigmented lesions: an ultrastructural study. *Trans Am Ophthalmol Soc* 1988; **86**:581–687.

6 Praetorius C, Sturm RA, Steingrimsson E. Sun-induced freckling: ephelides and solar lentigines. *Pigm Cell Melanoma Res* 2014; **27**:339–50.

7 Schwab C, Zalaudek I, Mayer C *et al.* New insights into oculodermal nevogenesis and proposal for a new iris nevus classification. *Br J Ophthalmol* 2015; **99**:644–9.

8 Rodriguez-Sains RS. Ocular findings in patients with dysplastic nevus syndrome. An update. *Dermatol Clin* 1991; **9**:723–8.

9 Toth-Molnar E, Olah J, Dobozy A, Hammer H. Ocular pigmented findings in patients with dysplastic naevus syndrome. *Melanoma Res* 2004; **14**:43–7.

10 Weis E, Shah CP, Lajous M *et al.* The association of cutaneous and iris nevi with uveal melanoma: a meta-analysis. *Ophthalmol* 2009; **116**:536–43e2.

11 Hurst EA, Harbour JW, Cornelius LA. Ocular melanoma - a review and the relationship to cutaneous melanoma. *Arch Dermatol* 2003; **139**:1067–73.

12 Kennedy C, Bajdik CD, Willemze R *et al.* The influence of painful sunburns and lifetime sun exposure on the risk of actinic keratoses, seborrheic warts, melanocytic nevi, atypical nevi, and skin cancer. *J Invest Dermatol* 2003; **120**:1087–93.

13 McGalliard JN, Johnston PB. A study of iris melanoma in Northern Ireland. *Br J Ophthalmol* 1989; **73**:591–5.

14 Bastiaens M, ter Huurne J, Gruis N *et al.* The melanocortin-1-receptor gene is the major freckle gene. *Hum Mol Genet* 2001; **10**:1701–8.

15 Baron AE, Asdigian NL, Gonzalez V *et al.* Interactions between ultraviolet light and MC1R and OCA2 variants are determinants of childhood nevus and freckle phenotypes. *Cancer Epidemiol Biomarkers Prev* 2014; **23**:2829–39.

16 Kvaskoff M, Whiteman DC, Zhao ZZ *et al.* Polymorphisms in nevus-associated genes MTAP, PLA2G6, and IRF4 and the risk of invasive cutaneous melanoma. *Twin Res Hum Genet* 2011; **14**:422–32.

17 Sturm RA, Duffy DL, Zhao ZZ *et al.* A single SNP in an evolutionary conserved region within intron 86 of the HERC2 gene determines human blue-brown eye color. *Am J Hum Genet* 2008; **82**:424–31.

18 Sturm RA, Fox C, McClenahan P *et al.* Phenotypic characterization of nevus and tumor patterns in MITF E318K mutation carrier melanoma patients. *J Invest Dermatol* 2014; **134**:141–9.

19 Daley GM, Duffy DL, Pflugfelder A *et al.* GSTP1 does not modify MC1R effects on melanoma risk. *Exp Dermatol* 2017; **26**:730–3.
20 Olsen CM, Green AC, Neale RE *et al.* Cohort profile: the QSkin Sun and Health Study. *Int J Epidemiol* 2012; **41**:929–929i.
21 Wilkerson CL, Syed NA, Fisher MR *et al.* Melanocytes and iris color. Light microscopic findings. *Arch Ophthalmol* 1996; **114**:437–42.
22 Sturm RA, Larsson M. Genetics of human iris colour and patterns. *Pigm Cell Melanoma Res* 2009; **22**:544–62.
23 Duffy DL. Sib-pair: a program for non-parametric linkage/association analysis. *Am J Hum Genet* 1997; **61**:A197–A.
24 R Foundation. *A Language and Environment for Statistical Computing*, 2014th edn. Vienna, Austria: R Foundation for Statistical Computing, 2016.
25 Duffy DL, Montgomery GW, Chen W *et al.* A three-single-nucleotide polymorphism haplotype in intron 1 of OCA2 explains most human eye-color variation. *Am J Hum Genet* 2007; **80**:241–52.
26 Andersen JD, Pietroni C, Johansen P *et al.* Importance of nonsynonymous OCA2 variants in human eye color prediction. *Mol Genet Genom Med* 2016; **4**:420–30.
27 Australian Institute of Health and Welfare. *Skin Cancer in Australia* (Cat. no. CAN 96). Canberra: AIHW, 2016.
28 Flaherty KT, Robert C, Hersey P *et al.* Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med* 2012; **367**:107–14.
29 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**:711–23.
30 Nordlund JJ, Kirkwood J, Forget BM *et al.* Demographic study of clinically atypical (dysplastic) nevi in patients with melanoma and comparison subjects. *Cancer Res* 1985; **45**:1855–61.
31 Schwab C, Mayer C, Zalaudek I *et al.* Iris freckles a potential biomarker for chronic sun damage. *Invest Ophthalmol Vis Sci* 2017; **58**: BIO174–9.
32 Olsen CM, Zens MS, Stukel TA *et al.* Nevus density and melanoma risk in women: a pooled analysis to test the divergent pathway hypothesis. *Int J Cancer* 2009; **124**:937–44.
33 Whiteman DC, Watt P, Purdie DM *et al.* Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. *J Natl Cancer Inst* 2003; **95**:806–12.
34 Vajdic CM, Kricker A, Giblin M *et al.* Sun exposure predicts risk of ocular melanoma in Australia. *Int J Cancer* 2002; **101**:175–82.
35 Mackie RM, English J, Aitchison TC *et al.* The number and distribution of benign pigmented moles (melanocytic naevi) in a healthy British-population. *Brit J Dermatol* 1985; **113**:167–74.
36 Zhu G, Duffy DL, Eldridge A *et al.* A major quantitative-trait locus for mole density is linked to the familial melanoma gene CDKN2A: a maximum-likelihood combined linkage and association analysis in twins and their sibs. *Am J Hum Genet* 1999; **65**:483–92.
37 Wachsmuth RC, Gaut RM, Barrett JH *et al.* Heritability and gene-environment interactions for melanocytic nevus density examined in a U.K. adolescent twin study. *J Invest Dermatol* 2001; **117**:348–52.
38 Lee S, Duffy DL, McClenahan P *et al.* Heritability of naevus patterns in an adult twin cohort from the Brisbane Twin Registry: a cross-sectional study. *Br J Dermatol* 2016; **174**:356–63.
39 Gandini S, Sera F, Cattaruzza MS *et al.* Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. *Eur J Cancer* 2005; **41**:2040–59.
40 Duffy DL, Zhu G, Li X *et al.* Novel pleiotropic risk loci for melanoma and nevus density implicate multiple biological pathways. *Nature Comm* 2018; in press.
41 Duffy DL, Iles MM, Glass D *et al.* IRF4 variants have age-specific effects on nevus count and predispose to melanoma. *Am J Hum Genet* 2010; **87**:6–16.
42 Chhabra Y, Yong HXL, Fane ME *et al.* Genetic variation in IRF4 expression modulates growth characteristics, tyrosinase expression and interferon-gamma responses in melanocytic cells. *Pigment Cell Melanoma Res* 2018; **31**:51–63.
43 Larsson M, Duffy DL, Zhu G *et al.* GWAS findings for human iris patterns: associations with variants in genes that influence normal neuronal pattern development. *Am J Hum Genet* 2011; **89**:334–43.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Iris colour correlation with peripupillary ring, visual eye colour and *OCA2* rs12913832 genotype.

Table S2 Linear regression model predicting log-transformed rater average of iris pigmented lesion count.

Table S3 Twin correlations for log-transformed observer mean iris pigmented lesion count.

Table S4 Candidate gene association results for log-transformed observer mean iris pigmented lesion count.

Fig S1. Rater agreement for iris pigmented lesion count between four observers (pairwise n = 945 maximum, 468 minimum).

Fig S2. (a) Tukey boxplot of the mean iris pigmented lesion count of up to four observers by iris colour rated by observer 1. (b) Tukey boxplot of the mean iris pigmented lesion count of up to four observers by skin colour on a three-point scale at clinical visit.

Fig S3. (a) Scatter plot of mean estimate iris pigmented lesion count (four observers) vs. total body naevus count ≥ 5 mm diameter. (b) Scatter plot regression lines for each iris colour, blue for blue/grey, green for green/hazel and brown for brown.

Fig S4. Upper panel: plot of mean iris pigmented lesion count; lower panel: proportion of each eye colour for in-person calls, blue for blue/grey, green for green/hazel and brown for brown.

Video S1 Author video.