Genomic Risk Score for Melanoma in a Prospective Study of Older Individuals

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Abstract

Background: Recent genome-wide association meta-analysis for melanoma doubled the number of previously identified variants. We assessed the performance of an updated polygenic risk score (PRS) in a population of older individuals, where melanoma incidence and cumulative ultraviolet radiation exposure is greatest.

Methods: We assessed a PRS for cutaneous melanoma comprising 55 variants in a prospective study of 12,712 individuals in the ASPirin in Reducing Events in the Elderly trial. We evaluated incident melanomas diagnosed during the trial and prevalent melanomas diagnosed pre-enrolment (self-reported). Multivariable models examined associations between PRS as a continuous variable (per standard deviation [SD]), and categorical (low-risk [0-20%], medium-risk [21-80%], high-risk [81-100%] groups) with incident melanoma. Logistic regression examined the association between PRS and prevalent melanoma.

Results: At baseline, mean participant age was 75 years; 55.0% were female, and 528 (4.2%) had prevalent melanomas. During follow-up (median = 4.7 years), 120 (1.0%) incident cutaneous melanomas occurred, 98 of which were in participants with no history. PRS was associated with incident melanoma (hazard ratio = 1.46 per SD, 95% confidence interval [CI] = 1.20-1.77) and prevalent melanoma (odds ratio [OR]=1.55 per SD, 95% CI = 1.42-1.69). Participants in the highest-risk PRS group had increased risk compared to the low-risk group for incident (OR=2.51, 95% CI = 1.28-4.92) and prevalent (OR=3.66, 95% CI = 2.69-5.05). When stratifying by sex, only males had an association between the PRS and incident melanoma, whereas both sexes had an association between the PRS and prevalent melanoma.

Conclusion: A genomic risk score is associated with melanoma risk in older individuals, and may contribute to targeted surveillance.

Melanoma incidence rises with age, and the majority of diagnoses occur in people aged 60 and older.[1,2] Age-specific incidence rates are highest among individuals aged ≥80, who represent the highest proportion of deaths from melanoma[2]. Therefore, the role of genetics in an older population has clinical relevance. Advancing age is associated with thicker melanomas, poorer disease-specific and overall survival, and greater recurrence risk.[3,4] Yet mechanisms underlying disease trajectory in older individuals are unclear. Melanomagenesis is a multi-step process resulting from interplay between environmental, host and genetic factors.[5] Understanding aetiological pathways underpinning pathogenesis, including the influence of heritable genes that may modulate risk, may help optimize surveillance and preventive strategies through more accurate risk assessment and stratification. Melanoma risk prediction models typically focus on phenotypic features, but have recently incorporated markers of genetic susceptibility, with the goal of improving risk stratification.[6-8]

Genome wide association studies (GWAS) provide insights into the biology of polygenic diseases such as melanoma, where risk is influenced by many common variants, or single nucleotide polymorphisms (SNPs) across the genome. While the contributions of individual SNPs to disease risk may be small, in combination they can have a considerable effect on risk, and therefore increased predictive value. This has led to the development of polygenic risk scores (PRSs), which aggregate the effects of SNPs across the genome into a single score.[9] Previous studies have attempted to predict risk using a PRS for melanoma[10-12] but have been limited by small numbers of identified variants at the time.[13-16] Many of these studies involved analyses of population-based cohorts with diversity of age, where incidence of melanoma overall is low[8,11,17] or familial melanoma cohorts where incidence is particularly high.[18,19] Melanoma PRS performance in older populations has not been evaluated.

The 'divergent pathways' of melanoma hypothesis describes melanoma development in older people as more UV dependent (less genetically-determined), compared with younger people, who require fewer UV insults to initiate tumorigenesis.[20] This hypothesis suggests that genetic factors play a greater role younger age groups. However, to our knowledge, no studies

have assessed a melanoma PRS in a cohort of older individuals, which is representative of a clinically relevant target population due to the higher risk of melanoma with age.

Recently, the largest ever GWAS meta-analysis of melanoma was published, identifying 56 loci associated with clinically confirmed melanoma at genome-wide significance ($P < 5 \times 10^{-8}$).[21] These newly-associated genetic variants can now be used to calculate an improved PRS for melanoma. Yet independent validation of this new PRS is challenging, given most of the large genetic studies of melanoma - including the UK Biobank - were used to derive the score within this meta-analysis. Further, many population-based cohorts do not contain large numbers of older individuals (aged >75 years) to model melanoma risk in the age range where the majority of melanomas occur. Therefore, it remains unclear the extent to which a PRS would be associated with melanoma in an older subgroup who would have high cumulative lifetime ultraviolet radiation (UV) exposure.

Here, we perform an independent validation of a newly-derived melanoma PRS in a well-characterised cohort of older individuals followed prospectively. The ASPirin in Reducing Events in the Elderly (ASPREE) trial population represents a large sample of healthy older individuals from Australia and the United States enrolled in an aspirin primary prevention trial.[22-24] All incident melanomas were adjudicated as secondary trial endpoints.[25] Our study helps assess the future clinical utility of genomic risk prediction for melanoma in older individuals.

Methods

Study design and population

The study population comprised genotyped participants of the ASPREE trial. Study design [26] and trial results[22,23] have been published previously. ASPREE was a randomised placebo-controlled clinical trial to determine whether daily aspirin extended disability-free survival in healthy older individuals with no history of diagnosed cardiovascular events, dementia or

physical disability at enrolment. Participants provided informed consent for genetic research. The study was approved by local Ethics Committees and registered on Clinicaltrials.gov (NCT01038583). Genotyping was performed on 14,052 participants, with a median follow-up time of 4.7 years (interquartile range = 2.1 years) per participant.

Endpoints

Endpoints used were primary invasive cutaneous melanoma and metastatic melanoma with unknown primary location occurring during the trial (incident). Metastatic recurrence was excluded. Incident melanomas were confirmed by expert panel using histopathology, imaging of metastasis or other clinical evidence. If a participant had two events during the trial, the time of the first event was used. Prevalent melanomas occurring pre-trial were self-reported by participants but not confirmed by review of medical records and assumed to be invasive. Age at diagnosis for self-reported melanomas was reported as either before or after 50 years.

Risk model, genotyping and polygenic score

Relevant phenotypic information from ASPREE included age, sex, and melanoma family history (parent/sibling/child). Information on skin pigmentation and number of nevi was not available. Genotyping was performed using the Axiom 2.0 Precision Medicine Diversity Research Array (Thermo Fisher, USA) following standard protocols. Variant calling used a custom pipeline aligned to hg38. We limited our study to participants with European ancestry to mitigate population stratification bias in polygenic scoring, given the PRS was derived from individuals of European descent.[21] To define genetic descent, we performed principal component analysis (PCA) using the 1000 Genomes reference population, excluding ASPREE samples that did not overlap the Non-Finnish European 1000 Genomes cluster (**Supplementary Figure 1**).[27] Samples from 12,712 participants passed the following filters: unrelated (identity-by-descent to third-degree relative); non-Finnish European descent;

minimum age at randomization 70 years. Of these, 12,081 (95%) were from Australian and 631 (5%) from US participants. Imputation was performed using the haplotype reference consortium, European samples (Michigan server).[28] Post-imputation QC removed variants with low imputation quality (r^2<0.3). We calculated PRS by using 56 variants associated with confirmed melanoma cases,[21] 55 of which were present in the ASPREE data (one low-quality imputation failure). Plink v1.9 calculated the weighted sum of the log odds ratios reported for the effect alleles for each variant. PRS SNPs and effect sizes are listed in **Supplementary Table 1**.

Statistical analyses

A multivariable Cox proportional hazards regression model was used to evaluate the association between PRS as a continuous variable with incident melanoma, reporting cause-specific hazard ratios (HR), adjusting for sex, melanoma family history, treatment (aspirin/placebo), age at enrolment, and interaction between PRS and treatment. In a separate model, PRS was categorized into three groups; low (0%-20%) medium (21%-80%) and high (81%-100%). We used Variance Inflation Factor (VIF) to check independence of terms in regression models. The discriminative ability of the model for incident melanoma was measured using the c-index. Competing risks estimates of the cumulative incidence were calculated and plotted for each group using the survfit function from the survival R package. Prevalence analysis of self-reported melanoma prior to enrolment used a logistic regression model, including sex and family history, reporting the odds ratio (OR) of the PRS. Goodness-of-fit for the logistic regression was assessed using the Tail-Based Max-test-statistic (TBM).[29] DeLong's test was used to compare between two correlated ROC curves for logistic regression.[30,31] Analyses were performed using R v3.6.1[32], tidyverse 1.3.0[33], survival 3.1.12[34], survminer 0.4.8 and pROC 1.16.2[35]. A z-test was used for the cox proportional hazards multivariable model, the logistic regression model and to determine if there were differences

between the PRS between age groups in which prevalent melanoma occurred. All statistical tests are two sided, and a p-value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics

The mean age of the 12,712 genotyped participants included in the analyses was 75 years (SD = 4.2), with the majority aged 70-79 years (**Table 1**). Overall, 6,990 (55.0%) participants were female, 391 (3.1%) were current smokers, and 528 (4.2%) self-reported a pre-enrolment melanoma. Of the prevalent cases, 98 participants (37 male, 61 female) reported melanoma prior to the age of 50 years. The PRS showed an approximately normal distribution in the population with mean -7.3 (standard deviation [SD] = 0.55) (**Supplementary Figure 2**). We scaled the PRS to have a mean of 0 (SD = 1) for the following analyses.

Association of PRS with incident melanoma

During follow-up, 120 (1.0%) incident cutaneous melanomas occurred, of which 110 participants were diagnosed with primary invasive melanoma, and 10 with metastatic melanoma with unknown primary location. Four participants had two melanomas diagnosed during follow-up. Twenty-two participants with incident cases also had a prevalent melanoma reported at enrolment. All prevalent cases were removed from the incidence analysis, leaving 98 cases (**Supplementary Table 2**).

PRS as a continuous variable in the model was associated with incident melanoma with a HR of 1.46 (95% CI = 1.20-1.77, p<0.001) per SD of the PRS. Female sex had a HR for melanoma of 0.49 (95% CI = 0.32-0.73, p<0.001) compared with men. The VIF for each term in the multivariable model was <1.1, indicating independence of the terms. The c-index for the model with PRS as a continuous variable was 0.643 (95% CI = 0.584-0.702), while the

c-index for the model excluding the PRS was 0.590 (95% CI = 0.530-0.648). We found no statistically significant interaction effect between aspirin treatment and PRS.

We further assessed the effect of the PRS by categorizing the PRS distribution into low- (1%-20%), medium- (21%-80%) and high-risk (81%-100%) groups. When considering associations with incident melanoma using the low-risk group as a reference, there was no statistically significant difference in risk of incident melanoma in the medium- and low-risk groups, but individuals in the high-risk PRS group had increased risk of incident melanoma versus the low-risk group (HR = 2.51, 95% CI = 1.28-4.92, p=0.007) (**Table 2**). **Supplementary Table 2** shows the distribution of incident cases by PRS group, stratified by sex. Individuals in the high-risk group had greater cumulative incidence than those in the low- and medium-risk groups (**Figure 1A**).

For males, both the medium- and high-risk PRS groups had greater risk of incident melanoma than the low-risk group (HR = 2.61 [95% CI = 1.03-6.63], p=0.04 and HR = 3.70 [95% CI = 1.37-9.98], p=0.009, respectively). For females, neither medium- or high-risk groups had statistically significantly higher melanoma incidence than the low risk group. Cumulative incidence of melanoma was higher in males than females when accounting for competing mortality risk (**Figure 1B-C**).

Association of PRS with prevalent melanoma

Prevalent (pre-enrolment) melanomas were self-reported as diagnosed at <50 or >50 years of age. No difference was observed in the distribution of PRS between these two age categories (z-test, p=0.40). The PRS was associated with prevalent melanoma when controlling for female sex, with a OR of 1.55 (95% CI = 1.42-1.69, p<0.001) per SD (**Table 3**). The TBM did not indicate a lack of goodness-of-fit (p>0.05). Individuals in the high-risk PRS group had a statistically significantly higher risk of prevalent melanoma versus the low-risk group (OR=3.66, 95% CI = 2.69-5.05, p<0.001). As with incident melanoma, females had

lower risk of prevalent melanoma compared with males (OR=0.82, 95% CI = 0.69-0.98, p=0.03).

Family history of melanoma was associated with increased melanoma prevalence (OR=2.36, 95% CI = 1.61-3.35, p<0.001) (**Table 2**). The AUC for the model with continuous PRS was 0.64 (95% CI = 0.62-0.66) which was a statistically significant improvement (p<0.001 DeLong test) compared to the model without the PRS, based only on family history and sex, for which the AUC was 0.54 (95% CI = 0.52-0.57). The ROC curve with PRS is shown in **Supplementary Figure 3**.

When stratifying the logistic regression by sex, the association between PRS and prevalent melanoma was statistically significant for both males and females; the OR in females was 1.72 (95% CI = 1.53-1.94) and in males was 1.39 (95% CI = 1.23-1.57). Prevalent melanoma is shown by PRS risk group and sex in **Supplementary Table 2**, and the multivariable logistic regression stratified by sex is shown in **Supplementary Table 3**.

Discussion

We assessed the performance of a polygenic risk score (PRS) for melanoma in a population of older individuals followed prospectively. We demonstrated a strong association between the PRS and incident melanoma risk in this cohort, and found meaningfully different rates of melanoma between low-, medium- and high-risk PRS groups. Our study highlights the potential use of genomic risk scores to improve the prediction of melanoma risk in older populations, where the burden of disease is particularly high. PRS may improve risk stratification for melanoma, towards targeted screening/surveillance for those at greatest risk. Our study population represents an age group at distinctly high-risk where the majority of melanoma incidence and mortality occur.[36] Our study validates a newly-derived PRS from a recent GWAS meta-analysis, confirming the PRS is statistically significantly associated with

incident and prevalent melanoma, even in a population of older individuals mostly residing in a country (Australia) with high ambient UV.

Previous studies have examined PRS performance in younger cohorts, using scores containing fewer SNPs, yet report similar performance for melanoma.[8,12,37,38] For example, a previous study examining the performance of a 45-SNP PRS in two population-based case-control studies from Australia and the UK, found the OR per SD of the PRS was 1.75 for Australia, and 1.63 for the UK.[8], and a 3-fold higher risk of melanoma for those in the highest versus lowest PRS tertile. These PRS effects are comparable with our findings (OR=1.5 per SD, 2.5-fold increase from low- to high-PRS). In another study, using a 21-SNP PRS involving 19,102 postmenopausal women from the Women's Health Initiative cohort, women in the highest tertile were 1.9 times more likely to develop melanoma compared to the lowest.[12] In our study, using a more recent PRS based on a larger number of SNPs, we have demonstrated a similarly robust association with incident melanoma in older adults. Our study has important clinical implications, providing evidence that genetic predisposition (rather than chronic UV damage alone) continues to play an important role in older age groups.

We observed a lower number of incident melanomas in females than males, consistent with previous reports[39,40] While female sex was protective in both incidence and prevalence analyses, the effect was stronger in the incidence analysis. Both sexes had statistically significant association of PRS with prevalent melanoma. Other studies have demonstrated sex differences in melanoma incidence, mortality and survival, with a female advantage generally reported.[41,42] Whether this stems from differences in hormonal factors, immunological responses, behavioural tendencies, genetics, or a combination of these, remains unclear. The low number of melanoma events in females in our study, however, does raise the possibility of power limitations in the assessment of PRS in females. Larger studies may reveal an association of the PRS for incident cases in females. In the self-reported prevalent cases, a retrospective evaluation of incidence across younger ages, the PRS calculated for females was higher than males (1.72 versus 1.39). Incidence of melanoma in

females is reported to exceed incidence in men up to the age of 49-50 years in the Australian population[43] which may contribute to the higher PRS score when self-reported cases including those <49 years are included. The extent to which sex differences in melanoma risk are driven by genetic factors warrants further investigation.

A strength of our study is the well-characterised, older population followed prospectively. All melanoma events that occurred during the ASPREE trial were adjudicated by an expert panel. Previous PRS studies have focused on examining younger cohorts and familial clusters. The median age at follow-up was 78 years, allowing observation of melanoma events in the most clinically relevant age group, with melanomas reported across the spectrum of close to a lifetime.

Limitations of our study include the unavailability of some key phenotypic and clinical risk factors associated with melanoma, such as UV exposure, naevi count and Fitzpatrick skin phototype which were not collected as part of the trial. The effects of incorporating our PRS into a conventional phenotype-based model, therefore, could not be assessed. Nevertheless, previous studies have shown a measurable increase in predictive ability of adding an earlier PRS to traditional melanoma risk factors, such as skin phototype.[8] A lack of efficacy towards the primary ASPREE endpoint led to early termination and a shorter follow-up period than originally intended, limiting the total number of melanoma events, especially in females. In addition, melanoma history prior to trial enrolment was self-reported and not verified through histopathology or supporting documentation, and the study only included participants who were relative healthy at age 70 years and older and hence excluded cases in the population whose melanoma progressed at younger ages. The reduced reliability of self-report for melanoma compared with other cancers[44] (e.g. due to confusion with basal and squamous cell carcinomas) may have contributed to an over-estimation of melanoma events. Our analysis only included participants of European genetic descent, limiting our ability to extrapolate findings to other ethnic backgrounds.

As our analysis focused on individuals in a country with high ambient UV, it would be informative to conduct a comparable PRS study in a cohort with a similar ethnic background, in a country with lower ambient UV. The melanoma PRS includes SNPs associated with a variety of biological processes, including pigmentary characteristics, naevus development, cell adhesion, immune regulation, and DNA repair.[21] Although differences in UV exposure may modify PRS performance across different populations, we note that many of the variants reported in the GWAS were unrelated to the classic cutaneous melanoma risk phenotypes (e.g. naevus count, hair colour, etc), and may not relate to UV exposure.[21] The exact mechanisms by which these SNPs influence melanoma development requires further functional analyses.

Currently, melanoma risk prediction models are largely based on phenotypic/clinical risk factors. To date, studies have shown that the addition of genomic risk scores based on previously identified SNPs to melanoma risk models containing traditional risk factors only modestly improves discriminatory power.[8] Such small incremental improvements offered by PRS may suggest only limited clinical potential. However, as our understanding of the genetic basis of melanoma evolves, and the performance of PRS improves, genetic testing may have an increasingly important role in future clinical practice for melanoma. Not only can a PRS potentially serve to improve risk prediction, risk-stratification and help personalise surveillance strategies, it can also shed light on the genetic architecture of melanoma, and enhance our understanding of the aetiological pathways underpinning melanomagenesis.

In conclusion, we present the first external validation of a newly-derived PRS for melanoma in a cohort of older individuals followed prospectively. Our study demonstrates that the PRS is a statistically significant discriminator of incident melanoma events in an older population, with potential clinical implications for risk-stratification, surveillance and prevention in this age group. Further studies are required to more rigorously assess the clinical utility of genetic risk scores for melanoma when combined with conventional risk phenotype information, and to determine the appropriate clinical context for their use.

Funding

This work was supported by an ASPREE Flagship cluster grant (including the Commonwealth Scientific and Industrial Research Organisation, Monash University, Menzies Research Institute, Australian National University, University of Melbourne); and grants (U01AG029824) from the National Institute on Aging and the National Cancer Institute at the National Institutes of Health, by grants (334047 and 1127060) from the National Health and Medical Research Council of Australia, and by Monash University and the Victorian Cancer Agency. AEC receives a NHMRC Career Development Fellowship (1147843). HPS holds an NHMRC MRFF Next Generation Clinical Researchers Program Practitioner Fellowship (APP1137127). VM is supported by an NHMRC Early Career Fellowship (1160757). PL is supported by a National Heart Foundation Future Leader Fellowship.

Notes

Role of the funder: No sponsor had any role in the study design, data collection, analysis, interpretation, the writing, and decision to submit the manuscript.

Author disclosures: HPS is a shareholder of MoleMap NZ Limited and E-derm Consult GmbH and undertakes teledermatological reporting for both companies. HPS is a Medical Consultant for Canfield Scientific Inc. and a Medical Advisor for First Derm and Revenio Research Oy. ATC has previously consulted for Bayer Pharma AG on aspirin-related topics. VM has received honoraria from Merck, Bristol-Myers-Squibb and Novartis. No other conflicts are declared for the other authors.

Author contributions: AB: Conceptualization, Data curation, Formal Analysis, Investigation, Software, Writing – original draft. **MY:** Data curation, Formal Analysis, Investigation, Writing – original draft. **MR:** Conceptualization, Data curation, Formal Analysis, Investigation, Software. **GP:** Data curation, Formal Analysis. **SGO:** Resources, Data curation, Investigation, Project

administration. **JT:** Investigation, Writing – original draft. **RW:** Data curation, Formal Analysis, Investigation. **AJ:** Conceptualization, Methodology. **YC:** Conceptualization, Methodology. **AML:** Conceptualization, Validation. **TY:** Methodology, Validation. **MJ:** Conceptualization, Validation. **HPS:** Conceptualization, Validation. **AEC:** Conceptualization, Validation. **MHL:** Conceptualization, Data curation. **PG:** Conceptualization, Resources, Data curation, Methodology. **CM:** Resources, Data curation, Formal Analysis, Methodology. **ATC:** Resources, Funding acquisition, Project administration, Supervision. **JJM:** Resources, Funding acquisition, Project administration, Supervision. **VJM:** Conceptualization, Methodology, Project administration, Supervision, Writing – original draft. **PL:** Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Writing – original draft. All authors were involved in writing (review and editing).

Acknowledgements: We thank the trial staff in Australia and the United States, the participants who volunteered for this trial, and the general practitioners and staff of the medical clinics who cared for the participants.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Tables

Table 1: Baseline characteristics of the ASPirin in Reducing Events in the Elderly trial population

Characteristic	No. (%)
Genotyped Participants, No.	12,712
Sex, Female	6990 (55.0)
Mean age at randomization (SD), years	75.06 (4.22)
Age Group	
70-74 years	7725 (60.8)
75-79 years	3198 (25.2)
80-84 years	1389 (10.9)
85+ years	400 (3.1)
Current or former smoker	5232 (41.2)
Diabetes	1178 (9.3)
Randomized to Aspirin	6340 (49.9)
Mean body mass index (SD), kg/m^2	27.97 (4.55)
Current alcohol consumer	10,132 (79.7)
Family history of melanoma	371 (2.9)
Mean Polygenic Risk Score (SD)	-7.3 (0.55)
Prevalent Melanoma (%) self-reported at enrolment	
None	12129 (95.8)
<49 years	98 (0.8)
50+ years	430 (3.4)

Table 2: Multivariable cox regression model for melanoma incidence in the ASPREE trial (clinically confirmed cases, excluding all prevalent cases)[a]

Covariate	PRS as Continuous Variable (per SD)		PRS as Categorical Variable (low, medium, high)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex (Female)	0.49 (0.32; 0.73)	<0.001	0.48 (0.35; 0.72)	<0.001
Family History*	1.57 (0.58; 4.29)	0.38	1.60 (0.62; 3.72)	0.36
Age at Enrolment	0.99 (0.95; 1.04)	0.83	1.00 (0.95; 1.04)	0.84
Treatment (Aspirin)	0.88 (0.59;1.30)	0.52	0.88 (0.59; 1.22)	0.53
PRS (per SD)	1.46 (1.20; 1.77)	<0.001	--	
Low PRS, 0%-20% (n=14)	--		1.00 (Reference)	
Medium PRS, 20%-80% (n=64)	--		1.61 (0.86; 2.99)	0.14
High PRS, 80%-100% (n=42)	--		2.51 (1.28; 4.92)	0.007

[a]120 incident melanomas occurred during the ASPirin in Reducing Events in the Elderly (ASPREE) trial, of which 98 had no pre-trial (prevalent) melanoma. 528 participants had self-reported prevalent melanoma at baseline. In total 626 participants had melanoma. CI = confidence interval; PRS = polygenic risk score, HR= hazard ratio.

Table 3. Logistic regression model for prevalent melanoma in participants enrolled in the ASPREE trial (pre-trial self-reported cases)[a]

Covariate	PRS as Continuous Variable (per SD)		PRS as Categorical Variable (low, medium, high)	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex (Female)	0.82 (0.69; 0.98)	0.03	0.82 (0.69; 0.98)	0.02
Family History	2.36 (1.61; 3.35)	<0.001	2.38 (1.63; 3.37)	<0.001
PRS (per SD)	1.55 (1.42; 1.69)	<0.001	--	
Low PRS, 0%-20% (n=64)	--		1.00 (Reference)	
Medium PRS, 20%-80% (n=349)	--		1.88 (1.41; 2.56)	<0.001
High PRS, 80%-100% (n=213)	--		3.66 (2.69; 5.05)	<0.001

[a]120 incident melanomas occurred during the ASPirin in Reducing Events in the Elderly (ASPREE) trial, of which 98 had no pre-trial (prevalent) melanoma. 528 participants had self-reported prevalent melanoma at baseline. In total 626 participants had melanoma. CI = confidence interval; PRS = polygenic risk score, OR= odds ratio.

Figure legend

Figure 1. Cumulative Incidence of primary cutaneous melanoma in (A) all participants, (B) male participants and (C) female participants. Competing risks model of the cumulative incidence of primary cutaneous melanoma in the ASPirin in Reducing Events in the Elderly (ASPREE cohort), stratified by a polygenic risk score (PRS) for cutaneous melanoma, categorized into low-risk (0%-20%); medium-risk (20%-80%), and high-risk (80%-100%) groups.