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Independent evaluation of melanoma polygenic risk scores in UK and Australian prospective cohorts

Supplementary Information

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Appendix 1: Supplementary Methods

UK Biobank

Study samples

This research has been conducted using data from UK Biobank, a major biomedical database (www.ukbiobank.ac.uk). A full description of the UK Biobank data has been reported previously.[1]

Participant characteristics

Self-reported ease of tanning was determined from the question "What would happen to your skin if it was repeatedly exposed to bright sunlight without any protection?", with response options "Never tan, only burn", "Get mildly or occasionally tanned", "Get moderately tanned", "Get very tanned", "Do not know", "Prefer not to answer" or missing value (data field 1727). We grouped "Do not know", "Prefer not to answer", and missing values into one category, referred to as "Not stated" (in the final dataset after quality control: n=7390 (1.9%), 219 (<0.1%), and 361 (<0.1%), respectively).
Self-reported ethnicity was provided by UK Biobank as determined from an amalgam of sequential branching questions (data field 21000).
The Townsend deprivation index was provided by UK Biobank (data field 189), based on participants' postcodes immediately prior to participant joining UK Biobank. Higher scores signify higher deprivation.

Cancer incidence data and death records

Participants gave permission for their health records to be accessed and for linkage to national cancer registries which record pathologically and clinically diagnosed cancers; for melanoma, essentially all diagnoses have pathological verification. Invasive melanoma incidence (International Classification of Diseases (ICD) code C43 for ICD10 and 172 for ICD9) was determined through linkage to cancer registry records (provided by NHS Digital

for England and Wales, and National Records of Scotland, NHS Central Register for Scotland).
Death records were provided by NHS Digital (for England and Wales) and the NHS Central Register (for Scotland).
The main outcome of interest in this study was the first incidence of invasive melanoma, so we censored participants at the first event of i) date of first diagnosis of invasive melanoma, ii) date of death, or iii) end of the follow-up period (31 March 2016 for England and Wales, and 31 October 2015 for Scotland).

Genotyping, imputation, and quality control

UK Biobank participants were genotyped using the UK BiLEVE Axiom Array (n~50,000) or the UKB Axiom Array (n~450,000). The UK Biobank dataset and the quality control and imputation approaches applied have been described elsewhere in detail.[1]
Within UK Biobank, biological samples were available for genetic analysis from 488,000 participants. The majority of participants were genotyped using a purpose designed UK Biobank Applied Biosystems Axiom array assessing 826,000 SNPs and indels. The quality control and imputation approaches applied have been described previously.[1]

UK Biobank provides lists of participants whose genetic results should be excluded on the basis of poor performance or close relatedness; these persons were excluded in our analysis. Non-European outliers were identified based on self-reported ethnicity and genetic principal components using an approach based on the UK Biobank definition of "Caucasian", but with one slight modification. We considered all participants who specified their ethnicity as white (whereas UK Biobank typically automatically exclude "Irish" and "any other white background"), then applied the 'aberrant' routine in R[2] to PCs 1&2, 3&4 and 5&6; the lambda parameter used was 100. This retained 397,430 individuals. We further excluded 76 participants due to revoked consent and 1707 participants with prevalent melanoma at baseline, yielding 395,647 participants with data available for analysis.

We used the --hardy 'midp' function in Plink v2 to calculate Hardy-Weinberg Equilibrium p-values for PRS SNPs based on imputed data. We note that the very large sample size yields small p-values with even small differences between the observed and expected number of heterozygote individuals (smallest observed $p=2.36\text{x}10^{-45}$ for rs7412746, observed het 48.0%, expected 49.2%). Thus, upon inspection, we did not exclude any variants based on small *p*-values. We confirmed all variants had minor allele count >100 in the dataset.

Finally, we obtained the imputation INFO score for all variants from UK Biobank resource 197 (https://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=1967, accessed 29/10/2021). All variants included in the PRS had INFO scores >0.78, with very high average score for each PRS indicating excellent quality of imputation (0.98 for PRS68 and PRS50 and 0.99 for PRS45).

Melbourne Collaborative Cohort Study

All MCCS participants provided informed consent and the Cancer Council Victoria Human Research Ethics Committee approved the study.[3]

Participant characteristics

Self-reported ease of tanning was determined from the question "What best describes what happens to your skin when, or if, you are exposed to strong sunshine?" with response options "I usually burn and rarely tan", "I burn first, then tan", "I usually tan and rarely burn". Self-reported ethnicity was determined from the question "Ethnic group(s)", with options "Australian", "New Zealander", "Greek", "Italian", "Maltese", "English", "Scottish", "Welsh", "Irish". We grouped these into categories as 1) Australian and New Zealander; 2) Greek, Italian, Maltese (abbreviated as "Greek/Italian"); 3) English, Welsh, Scottish, Irish (abbreviated as "British/Irish").

Cancer incidence data and death records

Incident melanomas (ICD10 code C43) were identified via linkage to the population-wide Victorian Cancer Registry and the Australian Cancer Database. Deaths were ascertained through record linkage to the Victorian Registry of Births, Deaths and Marriages, and the National Death Index at the Australian Institute of Health and Welfare.

The main outcome of interest was first incidence of invasive melanoma, so we censored participants at the first event of i) date of first diagnosis of invasive melanoma, ii) date of death, or iii) end of the follow-up period (31 June 2016 or 10 years after the second follow-up visit).

Genotyping, imputation, and quality control

Subcohort participants and additional participants with invasive melanoma were genotyped using the Illumina Infinium OncoArray-500k. Genotype imputation was done using the Michigan Imputation Server with the 1000 Genomes phase 3 data as reference panel.[4]

After imputation, we retained SNPs with imputation $r^2 \geq 0.3$.

Prior to quality control, data for 4,953 participants were available, of whom 4,710 were in the subcohort.

We excluded 24 participants who were ancestry outliers as identified using the FastPop method.[5]

To identify related individuals, we used the original post-QC genotype data, excluded SNPs with MAF<1% or Hardy-Weinberg Equilibrium ($p<0.0001$), pruned SNPs with LD $r^2>0.2$, and then calculated pairwise identity-by-descent between all pairs of individuals using Plink v1.9. This yielded 55 pairs of individuals estimated to be second- or first-degree relatives (PI_HAT>0.2; 110 unique individuals), and we excluded one individual from each pair at random.

We excluded 82 participants due to melanoma history prior to the baseline for this study (MCCS second follow-up visit). We also excluded 2 participants who were lost to follow-up due to migration <6 years after baseline, and 6 participants who were neither included in the subcohort nor had incident invasive melanoma in the 10-year follow-up period. We further excluded 19 participants with outlier values for genotype PCs 1-15 and 17-20 (>6 standard deviations difference to the mean). The variation along PC 16 was continuous and no clear outliers were identified; however, we carried out the exclusion as a sensitivity analysis, with similar results to the main analysis throughout (see below).

We used the --hardy 'midp' function in Plink v2 to calculate Hardy-Weinberg Equilibrium p-values for SNPs included in the PRS based on imputed data, restricting the analysis to n= 4528 individuals in the subcohort only.

We determined minor allele counts from dosage data.

We also compared the allele frequencies of minor alleles in the MCCS subcohort to the frequencies of the same alleles in UKB data, calculating the Pearson correlation separately for the variants included in PRS68, PRS50, and PRS45 (see Supplementary Results section below).

Finally, we checked that the average imputation r^2 for the variants included in the PRS was very high (0.92 for PRS68, 0.93 for PRS50 and 0.87 for PRS45).

Genome-wide association study meta-analysis

Analysis of the individual, contributing GWAS was unchanged from Landi *et al*.[6] The fixed-effect inverse variance weighted meta-analysis of log(OR) effect-sizes analysis was performed excluding both the confirmed melanoma and the self-report melanoma GWAS derived from UK Biobank. Resultant N following the GWAS of 20 confirmed melanoma GWAS and the 23andMe self-report GWAS was 31,459 cases and 353,984 controls.

In the full GWAS meta-analysis reported in Landi *et al.*,[6] 68 independent lead SNPs ($P < 5 \times 10^{-8}$) were identified in 54 loci. In the GWAS meta-analysis excluding UK Biobank participants, 50 of the 68 variants retained $p<5\times10^{-8}$ in the fixed effects meta-analysis (additionally requiring $p<5\times10^{-5}$ in the random-effects meta-analysis where $I^2>31\%$ as per Landi *et al.*;[6] Table S1).

23andMe GWAS summary statistics

Participants provided informed consent and participated in the research online, under a protocol approved by the external AAHRPP-accredited IRB, Ethical & Independent Review Services (E&I Review). Participants were included in the analysis on the basis of consent status as checked at the time data analyses were initiated.
The full GWAS summary statistics for the 23andMe discovery data set will be made available through 23andMe to qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Please visit https://research.23andme.com/collaborate/#dataset-access/ for more information and to apply to access the data.

Data sources for calculation of population-average absolute 10-year melanoma risk

Victoria

Age (5-year groups) and sex-specific population incidence and mortality rates were obtained from the Victorian Cancer Registry for the period 2009-2013.

Scotland

We obtained melanoma incidence and mortality data from Public Health Scotland (https://www.isdscotland.org/Health-Topics/Cancer/Cancer-Statistics/Skin/, accessed 2 September 2020), all-cause mortality data from the National Records of Scotland (https://www.nrscotland.gov.uk/statistics-and-data/statistics/statistics-by-theme/vital-events/deaths, accessed 2 September 2020), and mid-year population estimates from the UK Office for National Statistics

(https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/datasets/populationestimatesforukenglandandwalesscotlandandnorthernireland, accessed 2 September 2020).

England/Wales

We obtained melanoma incidence data for England from the UK Office for National Statistics (https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/datasets/cancerregistrationstatisticscancerregistrationstatisticsengland, accessed 2 September 2020), and for Wales from the Welsh Cancer Incidence and Surveillance Unit (http://www.wcisu.wales.nhs.uk/cancer-incidence-in-wales, accessed 2 September 2020).

For both England and Wales, we obtained melanoma and all-cause mortality data from the UK Office for National Statistics (https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/datasets/deathsregisteredinenglandandwalesseriesdrreferencetables, accessed 2 September 2020), and mid-year population estimates from the UK Office for National Statistics (https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/datasets/populationestimatesforukenglandandwalesscotlandandnorthernireland, accessed 2 September 2020).

Polygenic risk scores (PRS)

PRS45 had been previously evaluated in population-based case-control studies[7] and included 45 independent variants in 21 loci, of which 44 were genome-wide significant in genome-wide association studies[8] and one variant (*MITF* rs149617956) with robust association from whole-genome sequencing[9].

PRS68 included 68 independent genome-wide significant variants in 54 loci from the 2020 meta-analysis.[6] As this meta-analysis included UK Biobank samples, we also repeated the meta-analysis without UK Biobank samples. We then based PRS50 on the 50 of 68 variants that retained genome-wide significance, also taking forward the odds ratios from the meta-analysis without UK Biobank.

Calculation of genotype-specific relative risk scores

For all variants, we used pooled ORs from a fixed effects model meta-analysis, or a random effects model meta-analysis where there was evidence of heterogeneity ($I^2 \geq 31\%$).

We then followed a previously published approach[10] to determine genotype-specific relative risk scores for each variant as follows.

For a rare disease with log-additive risk model, a SNP with genotypes AA, AB, and BB and odds ratio OR_{SNP} for allele B vs allele A has genotype-specific relative risks of 1, OR_{SNP}, and OR_{SNP}^2. If allele B has frequency p_{SNP} in the population, then the genotypes AA, AB, and AB have frequencies $(1-p_{SNP})^2$, $2p_{SNP}(1-p_{SNP})$, and p_{SNP}^2 under Hardy–Weinberg equilibrium. Thus, the expected population average relative risk is $\mu_{SNP} = (1 - p_{SNP})^2 + 2p_{SNP}(1 - p_{SNP})OR_{SNP} + p_{SNP}^2 OR_{SNP}^2$. We then normalised the genotype-specific relative risks for each SNP by μ so that the expected average relative risk in the population would be 1, i.e. used the scaled relative risks $1/\mu_{SNP}$, OR_{SNP}/μ_{SNP}, and OR_{SNP}^2/μ_{SNP} for AA, AB, and BB genotypes, respectively.

For a participant with gene dosages d_{AA}, d_{AB} and d_{BB} for a given SNP, we obtained their SNP-specific relative risk as $d_{AA}/\mu_{SNP} + d_{AB}OR_{SNP}/\mu_{SNP} + d_{BB}OR_{SNP}^2/\mu_{SNP}$. Relative risks across SNPs were combined using a log-additive model to obtain a PRS-specific relative risk for each participant and each PRS. The normalisation approach also ensures the different PRS are on similar scales and comparisons between PRS are meaningful.

For variants in PRS45, the expected allele frequencies were obtained from controls in the original GWAS meta-analysis. For variants in PRS68, the expected allele frequencies were based on the HRC reference panel, as calculated in the recent meta-analysis (Supplementary Table 3 of the 2020 GWAS meta-analysis paper[6]). We also carried out a sensitivity analysis based on allele frequencies from gnomAD, which yielded highly similar normalisation factors (see below).

PRS normalisation factors using allele frequencies from gnomAD

To check the sensitivity of the genotype-weights with respect to allele frequencies in the reference population, we obtained allele frequencies for all PRS45 and PRS68 SNPs from gnomAD[11] v2.1.1, restricting the analysis to individuals who were not ascertained for having cancer in a cancer study (n=134,187), and with North-western European ancestry (n~4,250 for non-exonic and n~23,500 for exonic variants). Due to the small number of Southern European individuals (n~50 for non-exonic variants), we did not carry out a separate analysis based on allele frequencies in these individuals.

Population-average and PRS-adjusted absolute melanoma risks

As participants completed the baseline at different time points, the potential maximum follow-up time for participants was different. To account for this, we obtained the final absolute melanoma risk for each participant by linearly scaling the absolute 10-year risk (multiplying the risk by the number of years between the participant's recruitment and the end of the cancer incidence follow-up period and dividing by 10).

We obtained PRS-adjusted absolute melanoma risks for each participant and each PRS by multiplying the corresponding sex-and-age-specific final absolute risk (adjusted for the maximum possible follow-up time for the participant) by the participants' PRS-specific relative risk.

Association between PRS and melanoma incidence

The main fully-adjusted model included age, sex, self-reported ethnicity and ease of tanning, as well as the first 20 genetic principal components as covariates. We compared these results to unadjusted results from univariable models, as well as to results from partially-adjusted multivariable models that only included 1) age and sex; 2) age, sex, and self-reported ethnicity; 3) age, sex, self-reported ethnicity and ease of tanning.

For UKB, all analyses including self-reported ethnicity excluded 2 participants with missing values, and we carried out an additional analysis by extending the main model to include additional covariates: 1) skin colour and hair colour; 2) education and Townsend deprivation index; 3) skin colour, hair colour, education and Townsend deprivation index.

Moreover, we calculated association for PRS relative risk quintiles and separately, deciles, both using the 40-60th percentile as the reference category.

For UKB, PRS standard deviations (sd) were determined based on PRS values from all participants. For the MCCS, PRS standard deviations and the thresholds for PRS quintiles and deciles were determined based on the subcohort only.
In all analyses, we obtained 95% confidence intervals for subhazard ratio (SHR) estimates. For MCCS, we further verified the results using weighted Cox regression (Prentice model, R function "cch" in package "survival") which is designed for case-cohort studies, weighting data from subcohort participants by a factor of 1/0.22028 (where 0.22028 is the number of subcohort participants with final data included in the analysis (n=4,528) divided by the number of all participants who attended the second follow-up visit and did not have a prior diagnosis of invasive melanoma (n=20,556)).

Calibration

We evaluated calibration of unadjusted and PRS-adjusted absolute 10-year risks by comparing the expected ("*E*") and observed ("*O*") numbers of melanoma cases for each risk quintile ("*E/O*" ratio). For MCCS, we scaled up data from subcohort participants by a factor of 1/0.22028, and calculated robust standard errors for *E/O* to obtain 95% confidence intervals.[12] For UKB, we calculated 95% confidence intervals for *E/O* by assuming a Poisson distribution for *O*, as $E/O * \exp(\pm 1.96/\sqrt{O})$.[13] As a potential limitation, we note that the scaling factor would be different based on data before or after quality control, as participants of non-European ancestry were more likely to be excluded during quality control, and could have different melanoma risk.
For calibration by Townsend index in UKB, we categorised the Townsend deprivation index as quartiles based on the 395,647 participants after quality control and excluding 475 participants with missing Townsend deprivation index values. We then assessed calibration for each quartile of the Townsend deprivation index.

Discrimination

We calculated the AUC for the PRS relative risk, as well as the unadjusted and PRS-adjusted absolute risks using the R function "roc", with confidence intervals obtained using the function "ci" (both package "pROC"). The AUC ranges from 0 to 1, with 0.5 representing a completely random ranking and 1.0 perfect discrimination.

R^2 on the liability scale

We used the method described by Lee *et al.*[14] to convert the AUC values for the PRS relative risk, as well as the unadjusted and PRS-adjusted absolute risks, to the explained variance (R^2 on the liability scale). In particular, for a given AUC value, R^2 on the liability scale can be calculated as
$R^2 = 2*Q^2/[(m_2-m)^2+ Q^2\text{^}2*m*(m-t)+ m_2*(m_2-t)]$
where Q is the inverse of the cumulative density function of the normal distribution up to values of AUC, m is the mean liability for cases, m_2 is the mean liability for controls, and t is the threshold on the normal distribution that truncates the proportion of disease prevalence K.
Moreover, for a given disease prevalence K, m can be calculated as m=z/K, where z is the height of a normal density curve at the point according to K.
Finally, m2 can be calculated as m_2=-m*K/(1-K).

Therefore, the R^2 on the liability scale can be obtained directly from the AUC and the population prevalence K of a disease, which was assumed to be 1.5% as in Landi *et al.*[6] to allow for comparisons with previous work.

Estimated 10-year absolute risks by PRS quintile and age

We used the following approach to calculate estimates of 10-year absolute risks by PRS50 quintile and age. We selected PRS50 for this illustration as the underlying GWAS data were independent of both UKB and MCCS, and it had better performance than PRS45.

For males and females in England/Wales with European-ancestry and PRS50 in the top 20% of the distribution, the absolute risks for each age were approximated as

$$AR(sex, age) * SHR(top\ PRS\ quintile)$$

where $AR(sex, age)$ is the unadjusted absolute risk for the respective sex and age group based on population-wide data for England/Wales, $SHR(top\ PRS\ quintile)$ is the SHR for the top PRS50 quintile in UKB relative to the reference middle quintile (see Table S4). The absolute risks for males and females in Scotland were estimated analogously based on population-wide data for Scotland and association results in UKB. For males and females in Victoria, the absolute risks were estimated analogously based on population-wide data for Victoria and association results in MCCS. We followed the same approach to estimate absolute risks for other PRS50 quintiles.

We then calculated at which age males or females in the top or bottom 20% PRS50 would reach the same absolute risks as the population-average 50-year old of the same sex.

Sensitivity analyses

For UK Biobank, we carried out sensitivity analyses restricting to participants 1) with UK Biobank "Caucasian" flag and no "poor heterozygosity/missingness" flag (n=373,899); 2) recruited in England/Wales (n=365,449); 3) recruited in Scotland (n=30,198).

For the MCCS, we carried out sensitivity analyses restricting to 1) participants with 10 years of follow-up data (n=4,314); 2) participants within 6 standard deviations of the mean on genetic principal component 16 (n=4,699); 3) participants with self-reported Australian/New Zealand ethnicity (n=3,613). Characteristics of these participants subgroups are summarised in Table S8.

Appendix 1: Supplementary Results

Comparison of allele frequencies in UKB and the MCCS

For each of PRS68, PRS50 and PRS45, we found that the included variants had similar frequencies in the MCCS subcohort and UKB cohort data (Pearson r2 of 0.97-0.98 based on the minor allele in the MCCS subcohort, see Supplementary Methods and individual allele frequencies listed in Table S1).

Associations of melanoma PRS with participant characteristics including traditional melanoma risk factors

While participants' self-reported ethnicity was not significantly associated with the PRS in multivariable analyses, we found significant associations in univariable analyses ($p<0.0006$; Table S3): MCCS participants with south-European ethnicity had 0.6-0.7-fold lower mean

PRS compared to those with Australian/New Zealand ethnicity; UKB participants with Irish ethnicity had 1.1-fold higher mean PRS than those with British ethnicity, and those with White/Other white ethnicity had 0.96-fold lower mean PRS. Participants with non-European ancestry were excluded, so those who self-reported Australian/New Zealand ethnicity in MCCS includes individuals descended from European migrants.

Association of the melanoma PRS with melanoma incidence

In UKB, the subhazard ratio (SHR) per 1 standard deviation of PRS was generally higher for PRS68 than for PRS50, but with overlapping confidence intervals, e.g. fully-adjusted: PRS68 SHR=1.80 (95% confidence interval (CI) 1.71-1.88), PRS50 SHR=1.73 (95% CI 1.65-1.81). By contrast, the estimates for PRS68 and PRS50 were almost identical in the MCCS.
We also considered the association of PRS quintiles and deciles with melanoma incidence (Figure S2; Table S4).
With covariates as in the full model above, SHR estimates for the highest PRS decile compared to the 40-60% PRS percentiles were about 2.5-3.1 in UKB, and 1.5-2.5 in the MCCS (higher estimates for PRS68 and lower estimates for PRS45; Table S4).

Calibration of absolute melanoma risks

Analysing population-wide data from different calendar periods in the UK, we found that absolute melanoma risks by sex and 5-year age group have risen sharply in England/Wales, with some increases also observed in Scotland (Figure S3). For example, the estimated 10-year risk of melanoma incidence for 65-69 year old males in England/Wales was 0.31% (95% CI 0.30-0.32%) based on 2001-2005 data, but 0.62% (0.61-0.63%) based on 2011-2015 data (2-fold increase). Moreover, the risk increase in England/Wales was generally stronger for older age groups, with a 1.75-fold increase for males aged 60-64 and about 1.5-fold increases for males aged 45-49, 50-55 and 55-59 over the same period.
Thus, the calibration of absolute melanoma risk predicted for UKB depends on the calendar periods used to estimate sex-and-age specific risks from population-wide data. As described in the main text, the 2011-2015 period corresponds to the last five years of follow-up of UK Biobank participants, so the absolute risks from this period were used for further analysis.

Given the overall under-prediction of melanoma incidence in the cohort based on age and sex data alone, we also considered a linear re-calibration of absolute risks so that the number of expected cases would equal the number of observed cases, in order to assess any relative under- or over-estimation by absolute risk quintile when incorporating the PRS (Figure 2). Except for the PRS45, where we found a trend towards over-prediction of risks for the lowest quintile of PRS45-adjusted absolute risks in the MCCS, and a slight under-prediction of risks for the highest quintile of PRS45-adjusted absolute risks in UKB (Figure 2), 95% confidence intervals for all other PRS45, PRS50 and PRS68 quintile estimates included unity.

Discriminative ability of absolute melanoma risks with and without PRS

At a threshold of the top predicted risk decile, the PRS68- and PRS50-adjusted absolute risks had same specificity (90%) but higher sensitivity for predicting melanoma incidence in UKB compared to unadjusted risks based only on age and sex (sensitivity 26% vs 15%

respectively, Table S6). At equivalent thresholds in the MCCS, the PRS68- and PRS50-adjusted absolute risks had slightly higher specificity but lower sensitivity compared to unadjusted risks (91% versus 85% and ~24% versus 31%, respectively), resulting in higher positive and negative likelihood ratios (Table S6).

Explained variation on the liability scale

Assuming a population-wide melanoma prevalence of 1.5% for individuals aged 50-74,[6] PRS68 explains 5.3% (95%CI 4.4-6.2%) variation on the liability scale in UKB and 4.6% (2.9-6.7%) in the MCCS, with similar estimates for PRS50 (4.7% (4.0-5.6%) and 4.3% (2.6-6.4%), respectively), and a slight decrease for PRS45 (3.7% (3.0-4.4%) and 2.1% (1.0-3.6%), respectively, see Table S7). For comparison, the total heritability captured by all variants included in the 2020 meta-analysis was ~8.5% (5-12%).[6]

For the 10-year absolute risks, as per the discrimination analysis, the PRS-adjusted absolute risks explained significantly more variation than the unadjusted risks (e.g. PRS50-adjusted absolute risks: 6.2% (5.3-7.2%) in UKB and 7.0% (4.8-9.6%) in the MCCS; unadjusted risks: 1.4% (1.0-2.0%) in UKB and 3.0% (1.6-4.9%) in the MCCS, see Table S7).

Sensitivity analyses

PRS normalisation factors using allele frequencies from gnomAD

When substituting allele frequencies from North-western European individuals included in gnomAD into the genotype weights for PRS calculation, the resulting normalisation factors had very high correlations with the normalisation factors in the main analysis (Pearson r^2>0.995).

Association between PRS and participant characteristics

In UK Biobank, the associations between PRS and participants' characteristics were very similar when restricting the analysis to i) participants with UK Biobank "Caucasian" flag and no "poor heterozygosity/missingness" flag; or ii) those recruited in England/Wales only. When restricting the analysis to participants recruited in Scotland, the results were also very similar, with slightly attenuated association between genetic principal components and PRS.

In the MCCS, the associations between PRS and participants' characteristics were also very similar in all sensitivity analyses.

Association between PRS and melanoma incidence

The SHR estimates for the association between PRS and melanoma incidence were slightly attenuated when restricting the analysis to UK Biobank recruited in Scotland only, but with wider 95% confidence intervals that overlapped the estimates from the main analysis. The results of all other sensitivity analyses in UK Biobank and the MCCS were very similar to the main analyses. This included the weighted Cox cause-specific analysis of the MCCS data.

Calibration of absolute melanoma risks

The under-prediction of melanoma incidence in UK Biobank was attenuated when restricting the calibration analysis to participants recruited in Scotland only, with 95% confidence intervals for *E/O* including unity, e.g. PRS50-adjusted absolute risk: *E/O*=0.96

[95% CI 0.80-1.14] for participants recruited in Scotland only, compared to O/E=0.91 [95% CI 0.87-0.95] in the main analysis). In the MCCS, the under-prediction was stronger when restricting analysis to participants with self-reported Australian/New Zealand ethnicity only, with *E/O* estimates outside the 95% confidence intervals for *E/O* estimates from the main analysis. For example, for the PRS50-adjusted absolute risks, we estimated *E/O*=0.57 (95% CI 0.50-0.66) in this sensitivity analysis and *O/E*=0.67 (95% CI 0.59-0.77) in the main analysis. The results of all other sensitivity analyses were very similar to the main analysis.

Discrimination analysis

The results of all sensitivity analyses for the discrimination analyses were generally very similar to the results of the main analyses, with AUC estimates within the 95% confidence intervals from the main analysis. In UK Biobank, the AUC estimates for PRS relative risks and PRS-adjusted absolute risks were slightly lower in the analysis based on participants recruited in Scotland only, but with wide confidence intervals (e.g. PRS50-adjusted absolute risk AUC 0.66 (95% CI 0.62-0.71) for participants recruited in Scotland only, compared to AUC 0.68 (95% CI 0.67-0.69) in the main analysis based on the full UKB cohort).

Comparison of discrimination to previous externally validated melanoma risk models

A recent systematic review of melanoma risk prediction models identified 40 publications with 46 different models, of which only 6 publications included an external validation[15] (and only 5 included AUC estimates based on independent validation data).

Fortes *et al.* 2010 developed a model based on common nevi, skin and hair color, freckles, and sunburns in childhood; they found an AUC of 0.79 (95%CI 0.75-0.82) in the development data and 0.79 (95%CI 0.70-0.86) in an independent dataset,[16] although a later study in another dataset estimated a lower AUC of 0.68 (95%CI 0.64-0.73).[17]

Fang *et al.* 2013 constructed a PRS based on 11 genetic variants, with an AUC of 0.62 (95%CI 0.60-0.65), compared to an AUC of 0.64 (95%CI 0.61-0.66) for a model based on age, sex, pigmentation, and an AUC of 0.69 (95%CI 0.64-0.69) for a model incorporating the 11 genetic variants, age, sex and pigmentation.[18] However, no external validation of AUC was provided.

Davies *et al.* 2015 developed a model based on hair colour, skin type, freckling, family history of melanoma, total body nevus count, number of large (≥5mm) nevi on body, and history of sunburn.[19] This model had an AUC of 0.75 (95%CI 0.73-0.78) in an independent validation dataset. A later study found a similar AUC in another independent dataset (0.72, 95%CI 0.68-0.76).[17]

Vuong *et al.* 2016 developed a risk model based on hair colour, nevus density, first-degree family history of melanoma, previous non-melanoma skin cancer and lifetime sunbed use.[20] This model achieved an AUC of 0.70 (95%CI 0.67-0.73) in the development data, with lower AUCs in four independent validation datasets: 0.66 (95%CI 0.63-0.69), 0.67 (95%CI 0.65-0.70), 0.64 (95%CI 0.62-0.66), and 0.63 (95%CI 0.60-0.67).

Cust *et al.* 2018 developed a risk model based on hair color, skin color, eye color, freckling as an adult, skin photosensitivity, self-reported nevi, sunbed use, keratinocyte cancer personal history, first degree family history of melanoma, vacation sun exposure, and blistering sunburns as a child, age, sex, also fitting the city of recruitment for the study populations and European ancestry as variables; this model had an AUC of 0.72 (95%CI 0.69-0.75) in an Australian and 0.65 (95%CI 0.62-0.68) in a UK case-control study.[7] Adding a PRS

based on 45 genetic variants (PRS45 included in the current study) increased the AUC to 0.74 (95% 0.71-0.77; +0.023, p=0.003) and 0.68 (95%CI 0.65-0.71; +0.028, p=0.002), providing evidence that adding genomic risk information to traditional risk factors improves risk prediction.

Vuong *et al.* 2020 developed a model based on clinically assessed number of naevi ≥2mm in diameter on the whole body and solar lentigines on the upper back (a 6-level scale), as well as self-reported hair colour at age 18 years and personal history of keratinocyte cancer.[21] This model had an AUC of 0.79 (95%CI 0.76-0.83) in the development data and 0.73 (95%CI 0.70-0.75) in a validation dataset.

Finally, a recent study (not included in the systematic review) evaluated the AUC of six previously proposed models (including two of the above) in an independent dataset, and generally found lower AUCs than those reported in the original studies.[17] Except for one model with lower estimates, the 95%CIs for the weighted AUC on external validation for all models examined also overlapped the 0.68-0.69 AUC estimate for the PRS50-adjusted absolute risks reported in this study.

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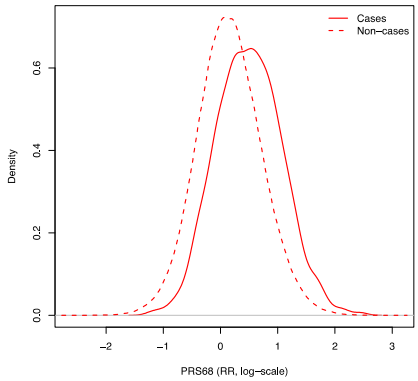
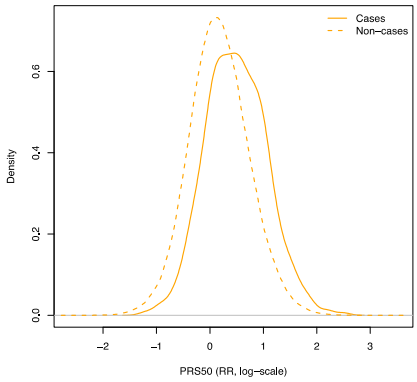
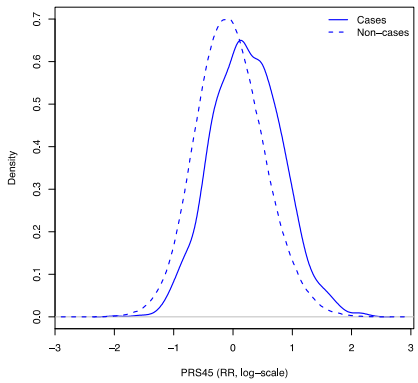
Supplementary Figures

(a) UKB

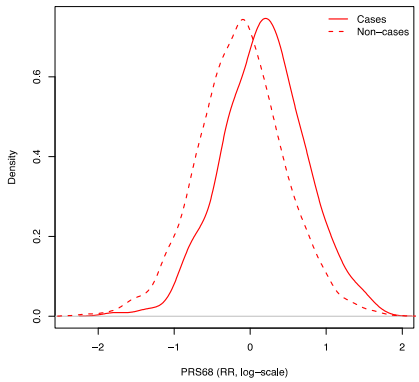


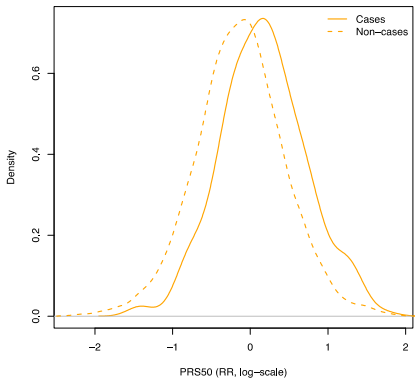


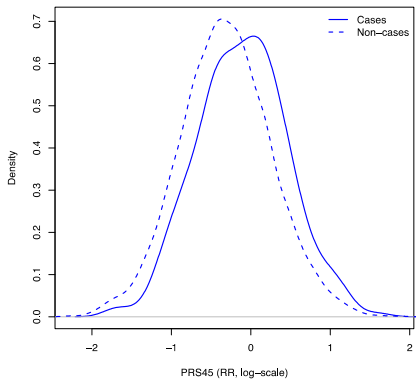


PRS (log)	PRS quantile					Mean	Sd
	0%	25%	50%	75%	100%		
PRS68	-2.66	-0.22	0.14	0.52	3.15	0.15	0.57
PRS50	-2.67	-0.20	0.16	0.53	3.55	0.17	0.56
PRS45	-2.78	-0.44	-0.07	0.32	2.83	-0.05	0.58

(b) MCCS



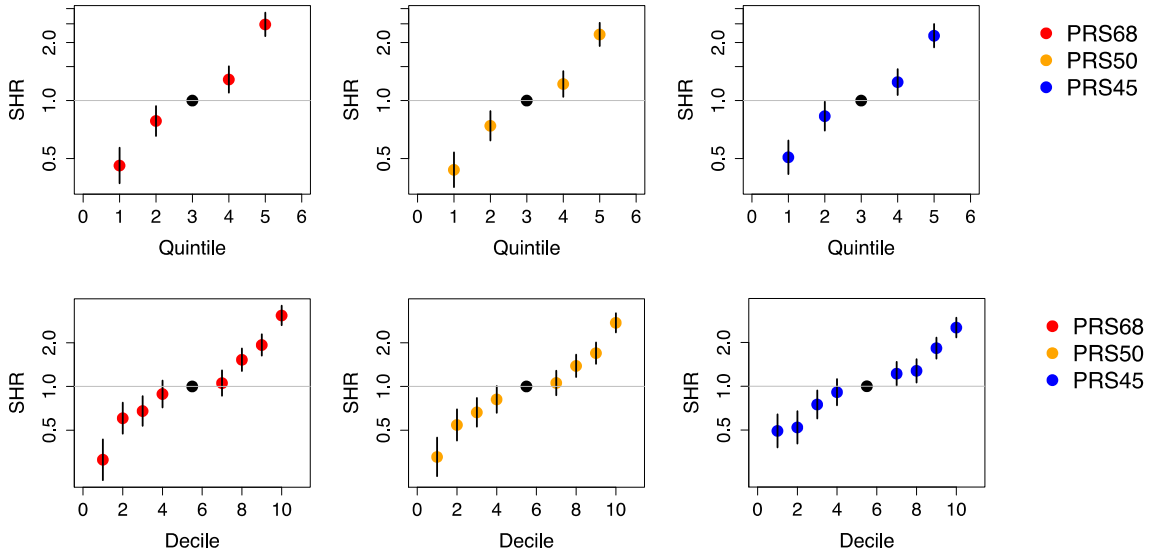




PRS (log)	PRS quantile					Mean	Sd
	0%	25%	50%	75%	100%		
PRS68	-2.39	-0.49	-0.12	0.25	1.98	-0.12	0.58
PRS50	-2.35	-0.49	-0.13	0.23	1.92	-0.13	0.58
PRS45	-2.30	-0.70	-0.32	0.06	1.89	-0.32	0.58

Figure S1. Distribution of melanoma PRS in (a) UKB and (b) MCCS participants, with statistics for the MCCS based on the subcohort only.

(a) UKB



(b) MCCS

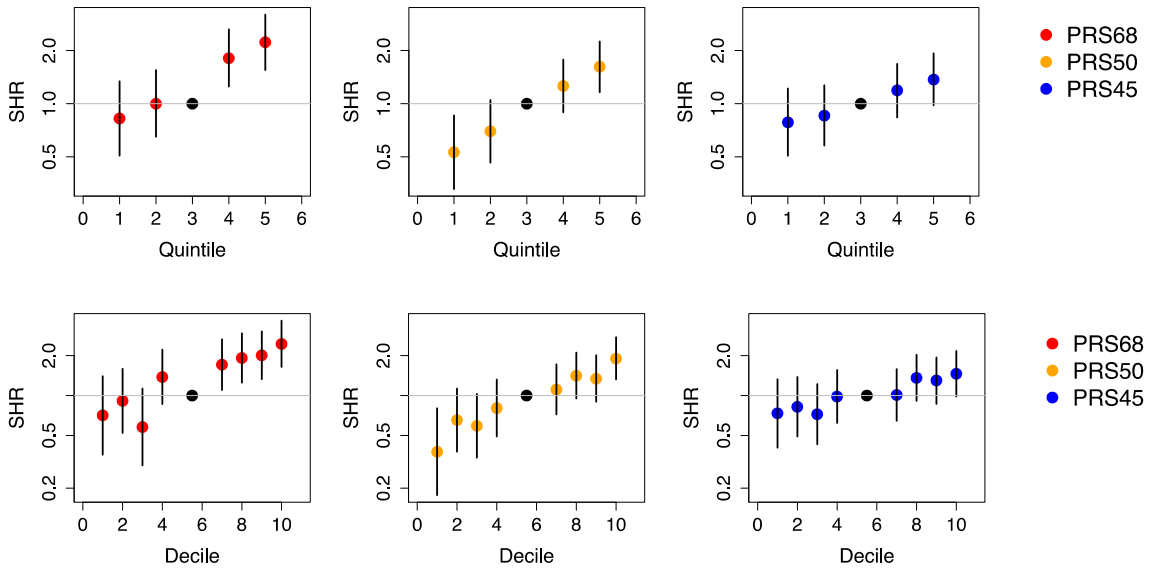


Figure S2. Association between melanoma PRS quintiles or deciles and melanoma incidence in (a) UKB and (b) the MCCS, with death as competing risk and adjustment for age, sex, self-reported ethnicity, ease of tanning and the top 20 genetic principal components. Estimates and p-values see Table S4.
SHR: subhazard ratio. Bars show 95% confidence intervals.

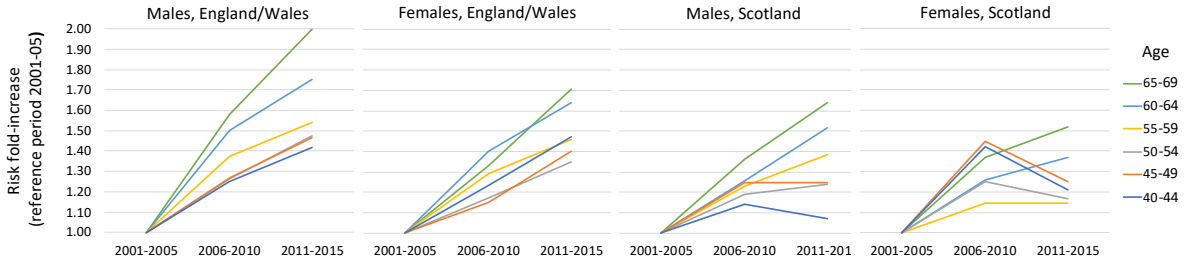
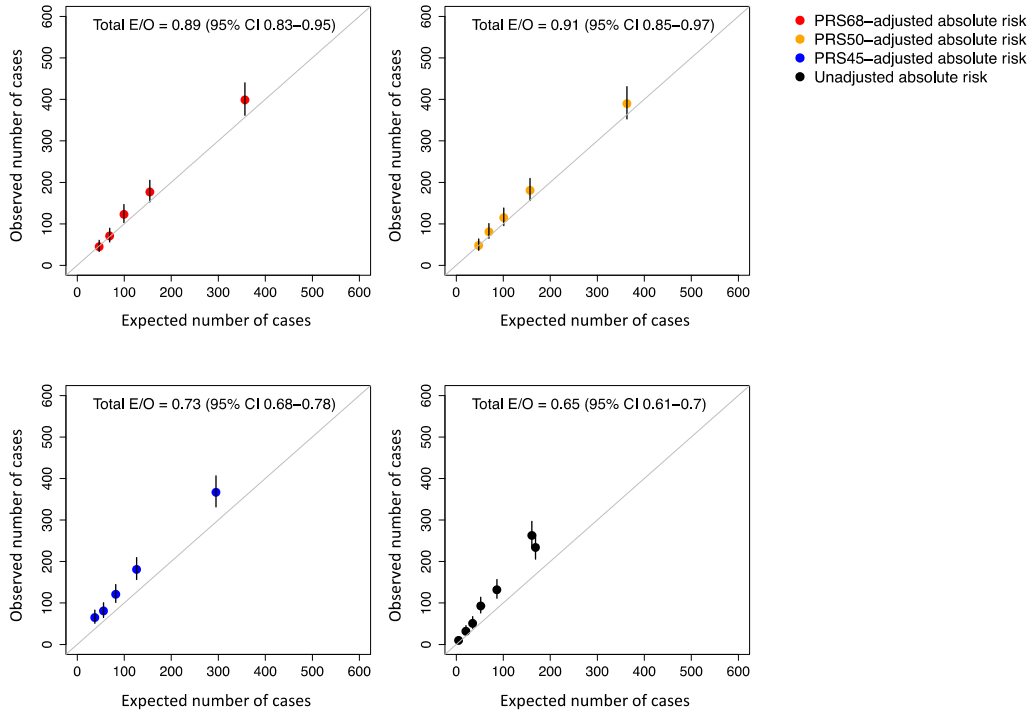


Figure S3. Relative increase in 10-year absolute melanoma risks calculated from cancer registry and population data for England/Wales and Scotland.

(a) UKB



(b) MCCS

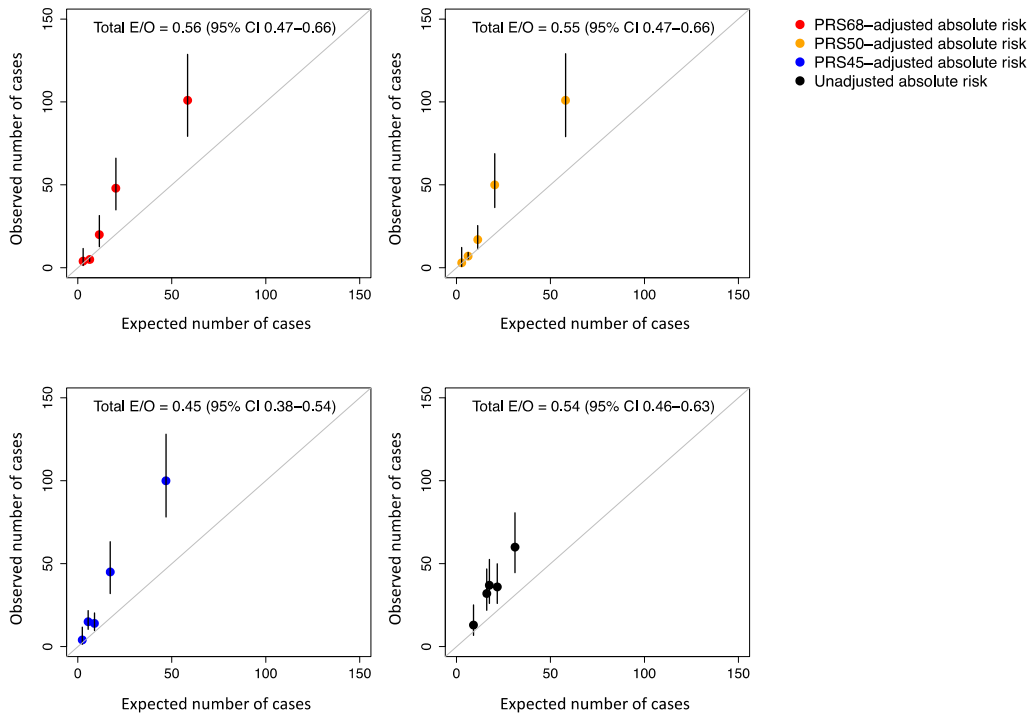
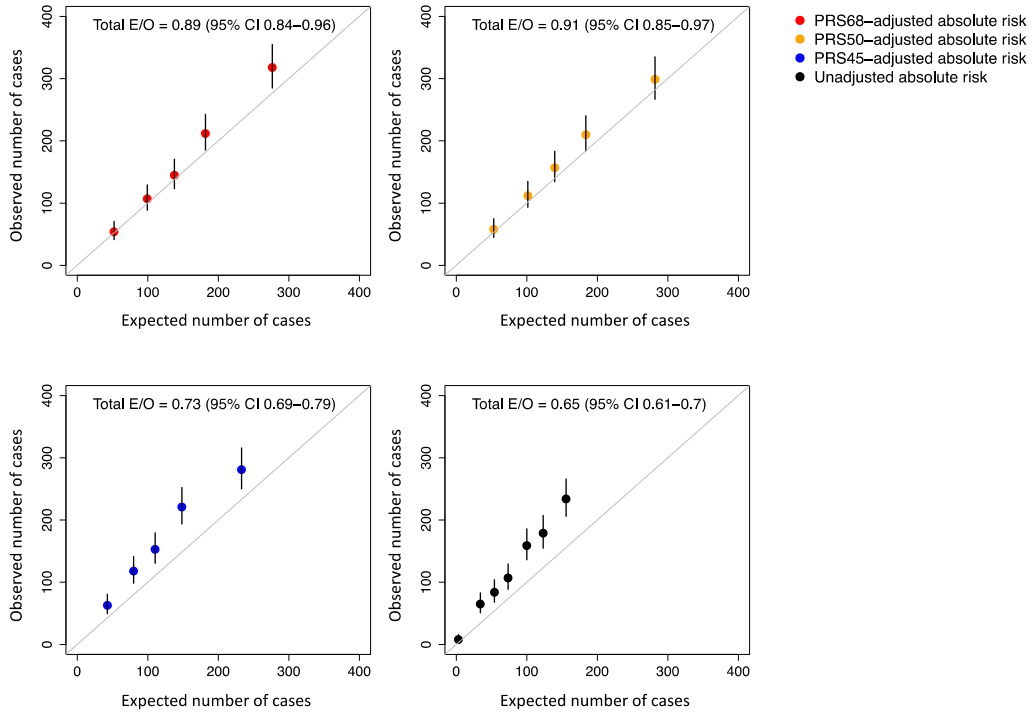


Figure S4. Calibration of absolute melanoma risks (by risk quintile) for male participants of (a) UKB and (b) the MCCS.
Bars show 95% confidence intervals.

(a) UKB



(b) MCCS

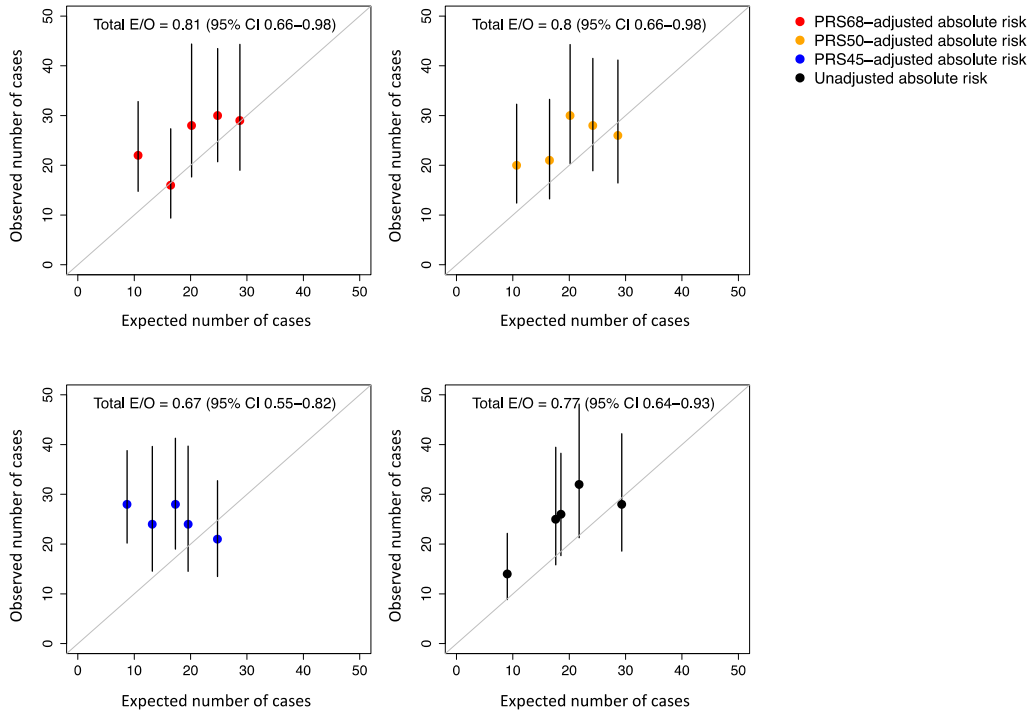


Figure S5. Calibration of absolute melanoma risks (by risk quintile) for female participants of (a) UKB and (b) the MCCS.
Bars show 95% confidence intervals.

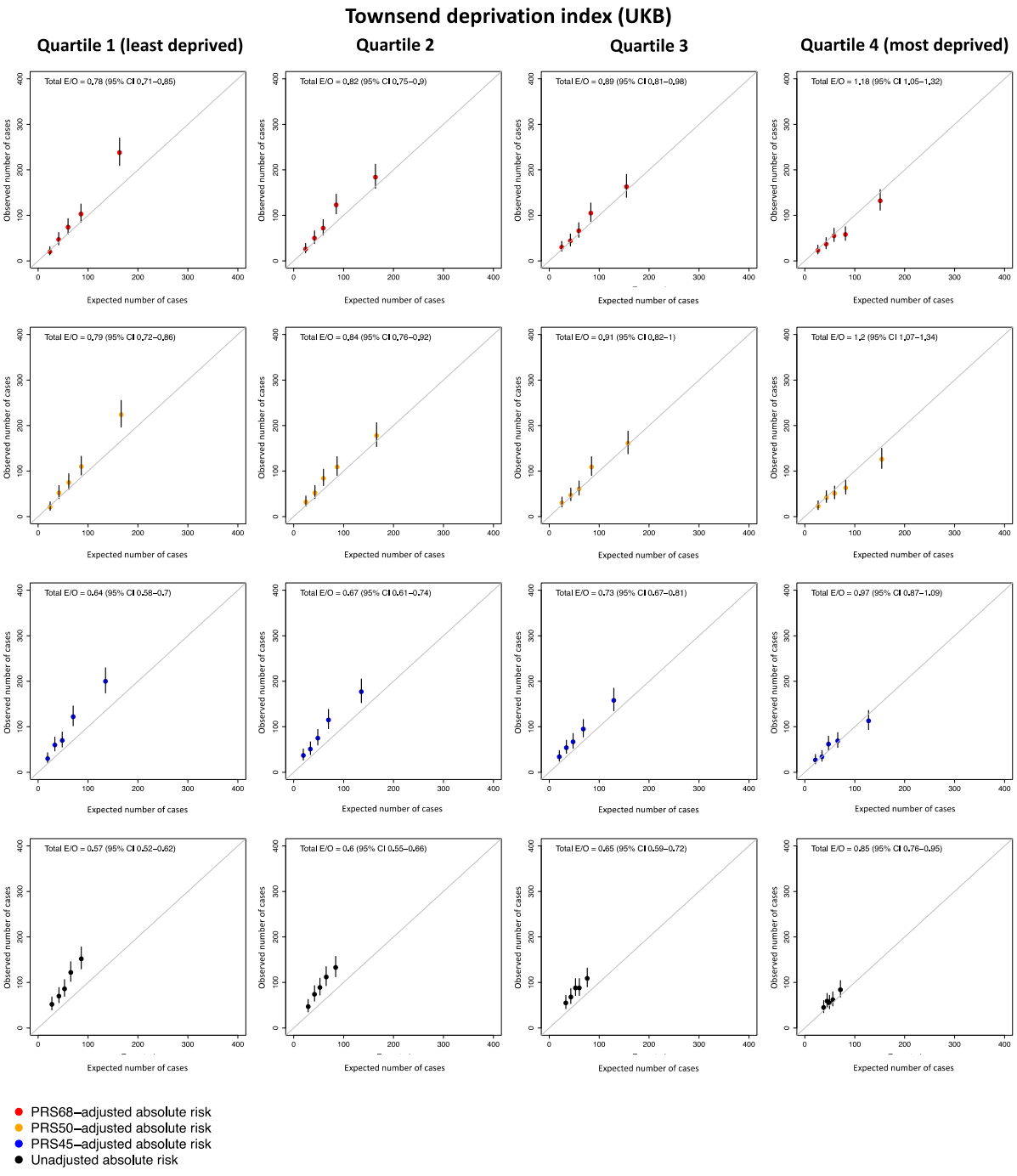


Figure S6. Calibration of absolute melanoma risks by risk quintile and Townsend deprivation index quartile in UKB.

Bars show 95% confidence intervals.

Supplementary Tables

Table S1. Variants included in the PRS, with odds ratios and allele frequencies.

Table S2. Associations between melanoma PRS and participants' characteristics including traditional melanoma risk factors: estimates for association with PRS relative risk (fold-difference on multiplicative scale) and their significance.

Table S3. Associations between melanoma PRS and participants' characteristics: results of multivariable and univariable sensitivity analyses.

Table S4. Subhazard ratios (SHR) and 95% confidence intervals (CI) for association between PRS quintiles or deciles and melanoma incidence in UK Biobank and the MCCS (reference category: 40%-60% PRS percentile), with death as competing risk and adjusting for age, sex, self-reported ethnicity and ease of tanning, and the top 20 genetic principal components.

Table S5. Calibration of age-and-sex-specific 10-year melanoma risks based on population-wide data from different periods.

Table S6. Sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio based on PRS-adjusted absolute risk thresholds.

Table S7. Variance (R^2) on the liability scale explained by the PRS relative risk, as well as the unadjusted and PRS-adjusted absolute risks.

Table S8. Characteristics of UK Biobank and MCCS participants included in sensitivity analyses (three sets each).

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