Genome-wide association study identifies three

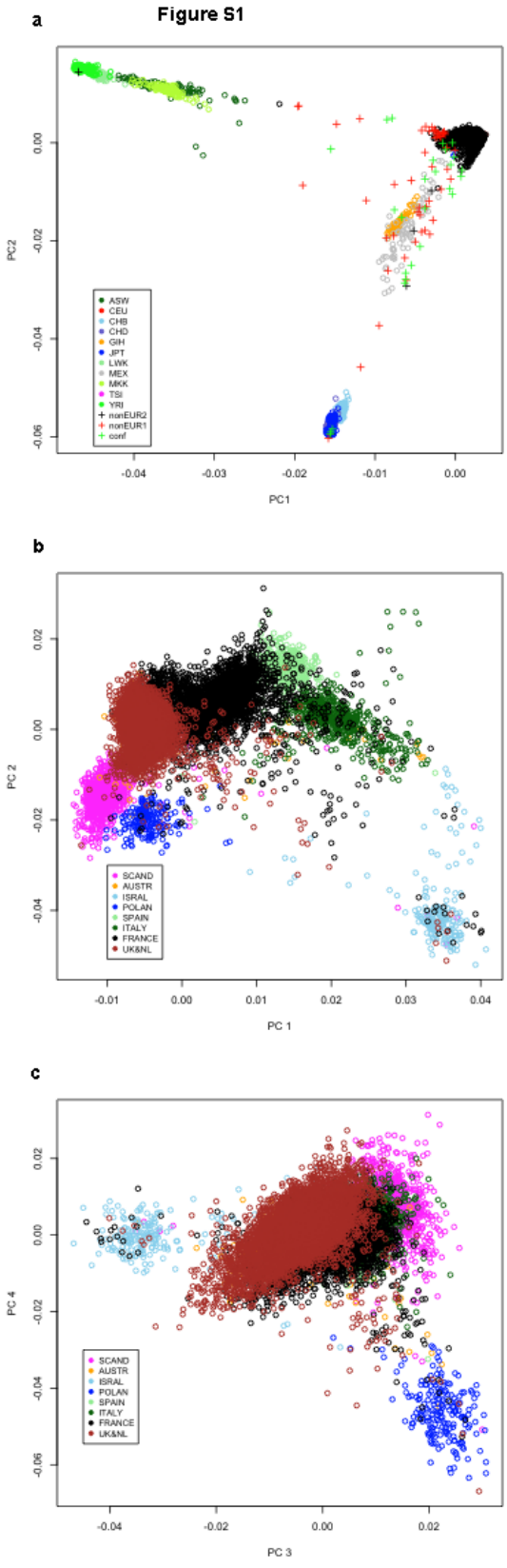
new melanoma susceptibility loci

Jennifer H Barrett, Mark M Iles, Mark Harland, John C Taylor, Joanne F Aitken *et al.* *

* A full list of author names appears in the main paper.

SUPPLEMENTARY INFORMATION

Supplementary Figures and Legends



Supplementary Figure 1 (see Figure S1):

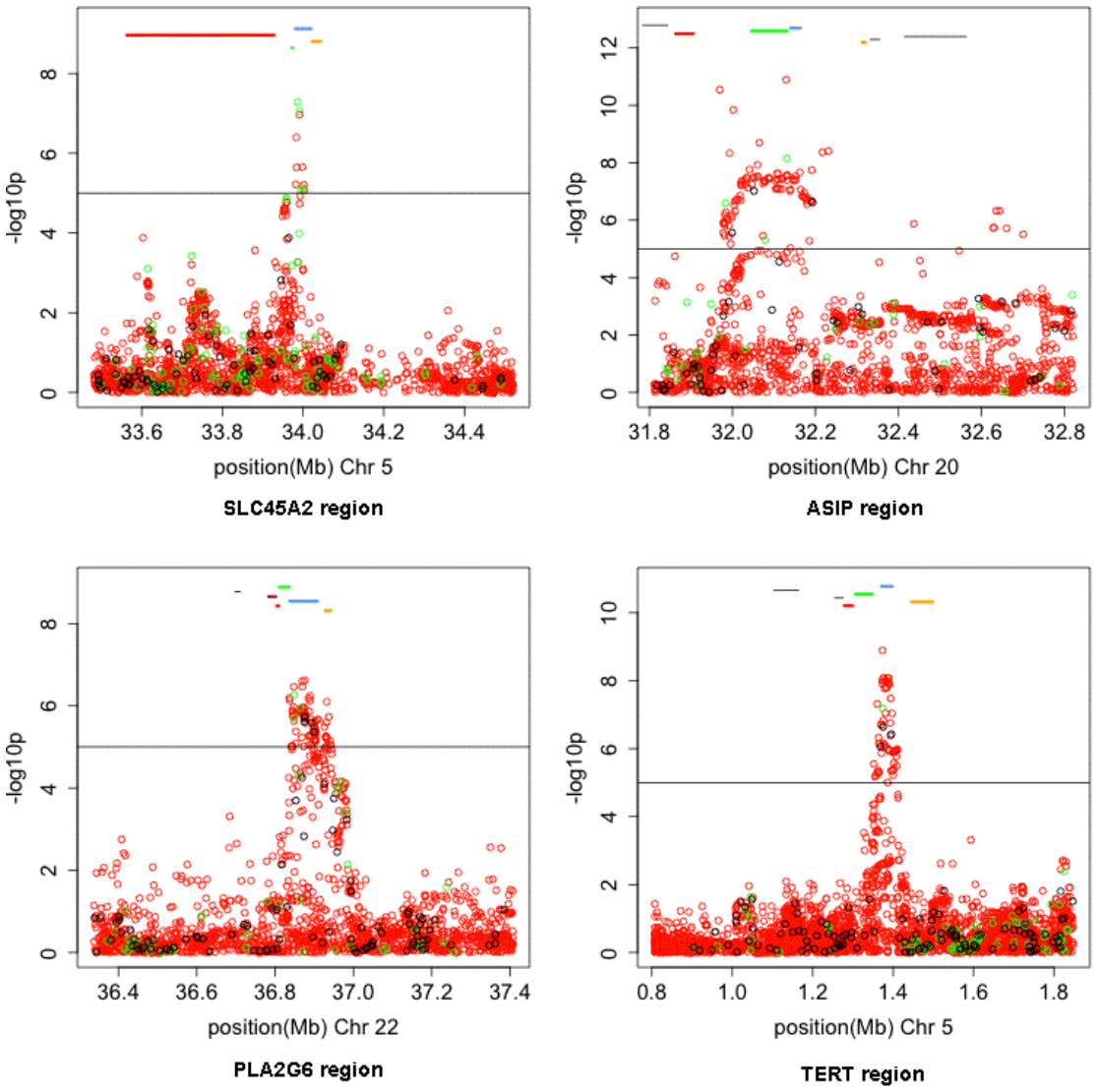
a) Principal Components for the genome-wide study combined with HapMap data. Plot of first two principal components from analysis of study data (after QC) combined with HapMap data. The ethnicity of the HapMap samples is indicated by color. The legend uses standard HapMap abbreviations (see http://hapmap.ncbi.nlm.nih.gov); briefly Chinese/Japanese samples are blue circles, African populations are green circles, European are red/magenta circles. Indian samples are yellow circles and Mexican grey circles. The GenoMEL samples declared to be of European ethnicity are black circles. Those GenoMEL samples that were excluded are represented by plus signs. Those colored green are samples we later confirmed to be non-European; those in red are from Phase 1 and those in black from Phase 2.

b & c) Plots of principal components 1 against 2 (b) and 3 against 4 (c) after QC for those GenoMEL samples declared to be of European ethnicity. Regions indicated by color: Scandinavia (magenta), Australia (orange), Israel (pale blue), Poland (dark blue), Spain (light green), Italy (dark green), France (black), UK and Netherlands (brown).

Supplementary Figure 2 (see Figure S2):

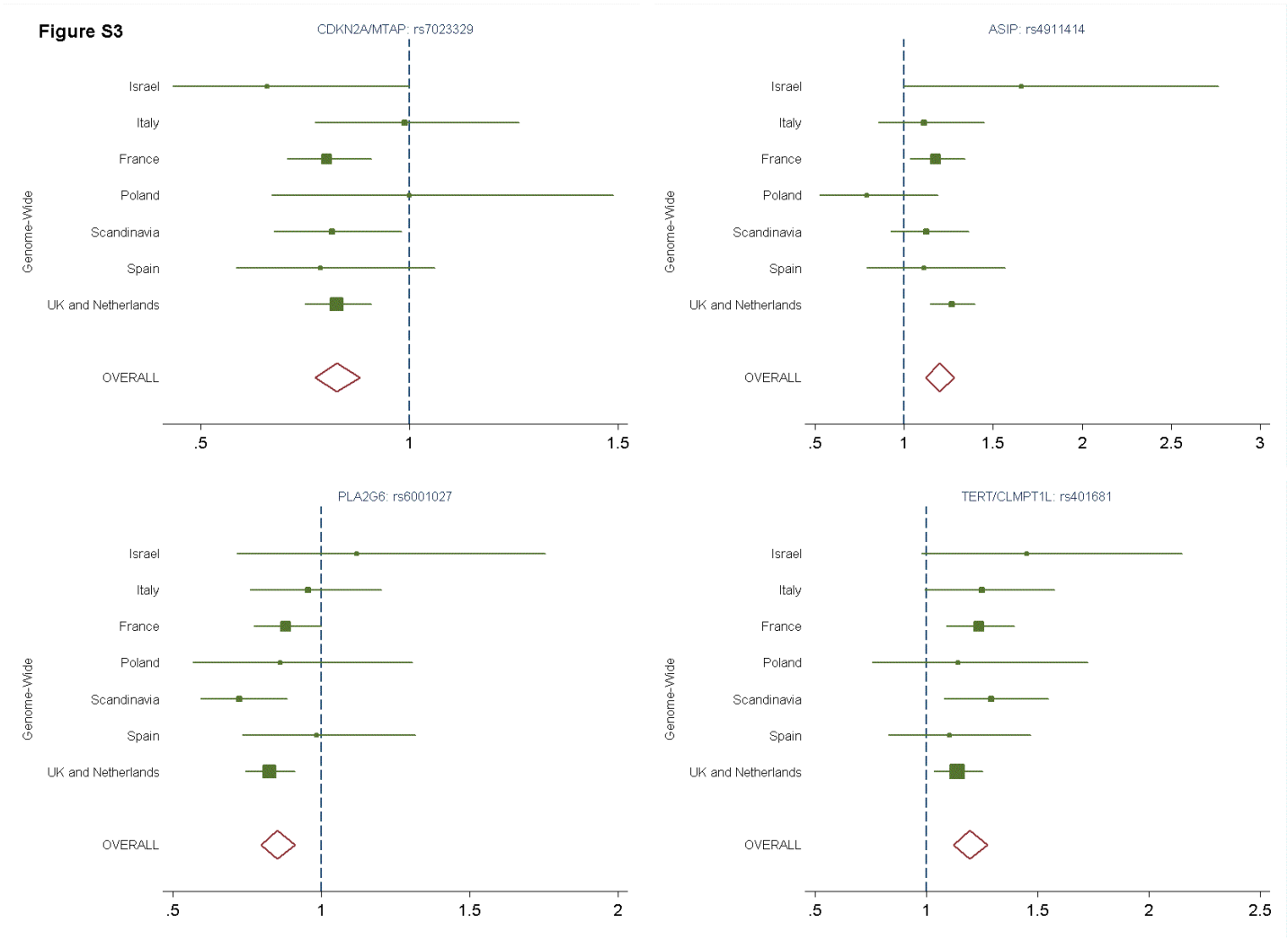
Stratified Cochran-Armitage (CA) trend tests for four known melanoma-susceptibility regions on chromosomes 5, 20 and 22. The $\log_{10}$ p-values are from the CA trend test (stratified by geographical region) for genotyped and imputed SNPs. SNPs genotyped for all samples are shown in black, SNPs imputed for all samples in red and SNPs genotyped for some samples and imputed for others (due to chip differences) in green. The solid horizontal line indicates a p-value of 10^{-5}. The horizontal lines at the top of the figure indicate the extent of genes in the central region of interest. In particular those lines that are colored (non-black) represent: (i) on chromosome 5, *ADAMTS12* (red), *RXFP3* (green), *SLC45A2* (blue), *AMACR* (orange), (ii) on chromosome 20, *CHMP4B* (red), *RALY* (green), *EIF2S2* (blue), *ASIP* (orange), (iii) on chromosome 22, *PICK1* (brown), *SLC16A8* (red), *BAIAP2L2* (green), *PLA2G6* (blue), *MAFF* (orange) and (iv) on chromosome 5, *SLC6A18* (red), *TERT* (green), *CLPTM1L* (blue), *SLC6A3* (orange).

Figure S2



Supplementary Figure 3 (see Figure S3):

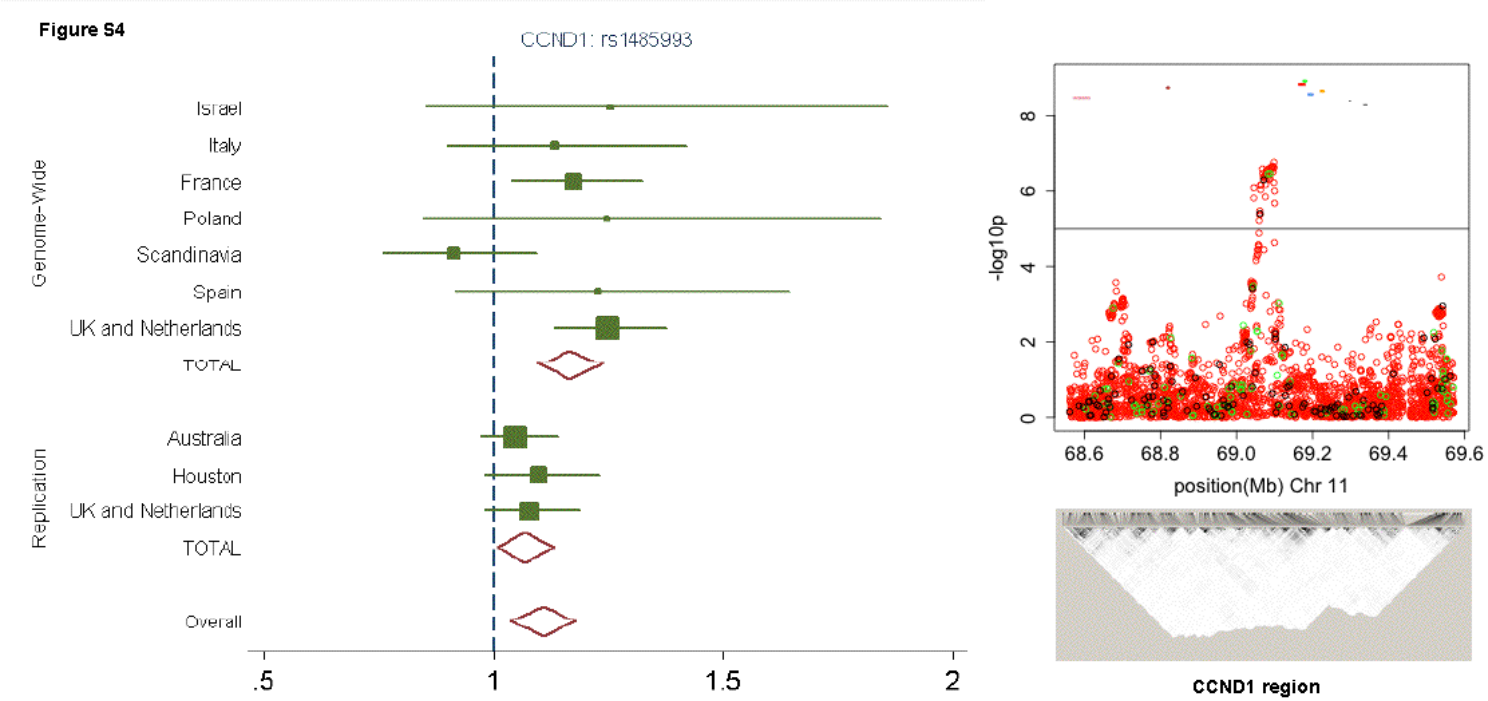
Forest plot of the per-allele OR for SNPs in 4 of the previously-known regions identified in the Phase 1 analysis, showing the current evidence for effects by geography in both the genome-wide data (Phases 1 and 2) and replication samples.



Supplementary Figure 4 (see Figure S4):

The left hand part of the figure is a forest plot of the per-allele ORs for the marginally-replicating region around *CCND1* on chromosome 11, showing the current evidence for effects by geography in the genome-wide and replication data.

The right hand part of the figure shows a plot of stratified Cochran-Armitage (CA) trend tests for the marginally replicating region around *CCND1* on chromosome 11. The $\log_{10}$ p-values are from the CA trend test (stratified by geographical region) for genotyped and imputed SNPs. SNPs genotyped for all samples are shown in black, SNPs imputed for all samples in red and SNPs genotyped for some samples and imputed for others (due to chip differences) in green. The solid horizontal line indicates a p-value of 10^{-5}. The horizontal lines at the top of the figure indicate the extent of genes in the region of interest. In particular those lines that are colored (non-black) represent *TPCN2* (pink), *MYEOV* (brown), *CCND1* (red), *FLJ42258* (green), *ORAOV1* (blue) and *FGF19* (orange). The greyscale plot indicates levels of pairwise linkage disequilibrium (measured by r^2) between SNPs, estimated from HapMap data using Haploview.[1]



Supplementary Tables

Supplementary Table 1:

Description of genome-wide samples. In total samples from 2,804 cases and 7,618 controls were included in the genome-wide analysis. Summary information detailing samples contributed, genotyping laboratories and phase of study is given for participating GenoMEL groups. Also listed are the numbers of samples genotyped by phase, the numbers excluded after quality control, and the remaining numbers of cases and controls. The genotyping laboratory is either SXS (ServiceXS, Leiden, The Netherlands), CNG (Centre National de Génotypage, Evry, France), or SAN (Sanger Centre, Cambridge, UK). 387 Australian samples (most of which pass QC) are listed as being in Phase 1, but are excluded from the total number after QC and from our analysis, because many are in the Australian replication set.

			Genotyped samples		Excluded samples		Samples in final statistical analysis			
Group	**Country**	**Lab**	**Phase 1**	**Phase 2**	**Phase 1**	**Phase 2**	**Total**	**% of genotyped**	**Cases**	**Controls**
Brisbane*	Australia	SXS	191	0	20	0	0	0%	0	0
Sydney*	Australia	SXS	196	0	17	0	0	0%	0	0
Paris	France	CNG	477	0	18	0	459	96%	459	0
Paris	France	SXS	197	135	24	11	297	89%	212	85
Tel Aviv	Israel	SXS	0	216	0	29	187	87%	112	75
Emilia-Romagna	Italy	SXS	200	0	11	0	189	95%	96	93
Genoa	Italy	SXS	198	192	13	14	363	93%	179	184
Leiden	Netherlands	SXS	199	199	9	6	383	96%	195	188
Bergen-Oslo	Norway	SXS	0	397	0	9	388	98%	194	194
Szczecin	Poland	SXS	0	195	0	7	188	96%	96	92
Barcelona	Spain	SXS	199	195	39	13	342	87%	164	178
Lund	Sweden	SXS	200	0	4	0	196	98%	99	97
Stockholm	Sweden	SXS	193	204	29	19	349	88%	164	185
Glasgow	UK	SXS	0	163	0	23	140	86%	75	65
Leeds	UK	CNG	91	0	13	0	78	86%	78	0
Leeds	UK	SXS	374	739	15	18	1080	97%	681	399
TOTAL GenoMEL			**2715**	**2635**	**212**	**149**	**4989**	**93%**	**2804**	**1835**
Other Control Samples										
French Controls	France	CNG	1824	364	282	1	1905	87%	0	1905
WTCCC 1958 Birth cohort	UK	SAN	1333	1512	103	133	2609	92%	0	2609
WTCCC NBS controls	UK	SAN	0	1393	0	124	1269	91%	0	1269
TOTAL other control samples			**3157**	**3269**	**385**	**258**	**5783**	**90%**	**0**	**5783**
TOTAL Samples			**5872**	**5904**	**597**	**407**	**10772**	**91%**	**2804**	**7618**

*** Samples included in first phase GWA but excluded from overall analysis because of overlap with other studies**

Supplementary Table 2:

Summary of SNPs and loci previously identified as associated with melanoma risk either in genome-wide association studies or in candidate gene studies. For the genome-wide studies, Bishop et al.[2] showed evidence for SNPs in the region of *MC1R* and *TYR* (both pigmentation genes). Further candidate gene studies focused on other loci associated with pigmentation. Brown et al.[3] showed evidence for a melanoma locus on chromosome 20 in the vicinity of the pigmentation gene *ASIP*. The nevus loci were identified in genome-wide studies of melanoma or nevi[4,5]. Data provided in this Table are taken from examination of the cumulative evidence from all cases and controls genotyped in the genome-wide component of this analysis. The *TERT/CLMPT1L* locus was identified as being associated with a number of cancers and was shown to be associated with melanoma risk in a candidate SNP analysis[6,7]. Further details of these results are in the text.

Chromosomal region	Candidate Gene	SNP/variant	Minor allele	MAF	Per allele OR for melanoma in this study (95% CI)	p	Reference Numbers	Melanoma-associated phenotype
5p15.33	*TERT/CLMPT1L*	rs401681	A	0.46	1.20 (1.12, 1.28)	2.98×10^{-8}	5	None known
5p13.2	*SLC45A2*	rs35390	C	0.02	0.36 (0.23, 0.53)	2.38×10^{-7}	6, 7	Pigmentation (black/blond Hair)
6p25-p23	*IRF4*	rs12203592	A	0.19	0.94 (0.84, 1.06)	0.32	10	Pigmentation (darker skin) and nevus count
		rs872071	A	0.5	1.07 (1.00, 1.14)	0.04	10	
9p21	*CDKN2A/MTAP*	rs7023329	G	0.49	0.83 (0.78, 0.88)	7.35×10^{-9}	1, 11	Nevus count
11q14-q21	*TYR*	rs1393350	A	0.28	1.30 (1.21, 1.39)	1.77×10^{-13}	1	Pigmentation (blond hair) & tanning response
16q24.3	*MC1R*	rs258322	A	0.11	1.70 (1.54, 1.87)	2.70×10^{-27}	1	Pigmentation (red hair) & sun sensitivity
20q11.2-q12	*ASIP*	rs17305657	A	0.1	1.29 (1.11, 1.49)	0.00068	13	Pigmentation (red hair and fair skin)
		rs2284378	G	0.33	1.21 (1.13, 1.29)	1.18×10^{-7}		
		rs4911414	C	0.34	1.20 (1.12, 1.28)	1.62×10^{-7}	8, 14	
		rs1015362	G	0.27	1.03 (0.96, 1.11)	0.40	8, 14	
		rs4911442	A	0.14	1.29 (1.14, 1.46)	0.000086	13	
22q13.1	*PLA2G6*	rs6001027	G	0.35	0.85 (0.79, 0.91)	2.23×10^{-6}	1, 11	Nevus count

Supplementary Table 3:

Detailed results from this study for the 7 regions targeted for replication, listing each SNP under consideration, their position and minor allele frequency (MAF); the per-allele OR and p-value are given for the genome-wide study presented here and for the replication datasets. For each part of the study results based only on genotype data are in bold, while those including any imputed data are in plain font. The Houston samples were genotyped on the Illumina OMNI array, so any SNP not on this array is entirely imputed. The Australian samples were genotyped on one of two arrays so all SNPs are genotyped on some samples and imputed in others: here the genotyped column includes only those samples that were genotyped for the SNP, while the imputed column includes all genotyped and imputed samples. For SNPs with positive support from the GWA replication data further genotyping was conducted in samples from the UK and Netherlands. Results are shown for the stratified analysis of the combined samples from the UK and Netherlands. Results of the fixed effects meta-analysis of the combined replication samples and the combined genome-wide and replication analyses are given for both genotyped data only and for genotyped and imputed data combined. Finally the results of the random effects meta-analysis and heterogeneity statistics are given for the combined genome-wide and replication analyses.

					Hypothesis Generating		**Replicating datasets**																	
					GenoMEL		**MDA, Houston**		**Australia (genotyped)**		**Australia (genotyped and imputed) PC corr**		**UK and Netherlands**		**Replication samples (genotyped)**		**Replication samples (genotyped + imputed)**		**Genome-wide plus Replication samples (genotyped)**		**Genomel-wide plus replication samples (genotyped + imputed)**		**Genomel-wide plus replication samples (genotyped + imputed) Random Effects**		**Heterogeneity**	
SNP	**Chromosome**	**Coordinate**	**Minor allele**	**MAF**	OR	p	OR	p	OR	p	OR	p	OR	p	OR and 95% CI	P-value	OR and 95% CI	P-value	OR and 95% CI	P-value	OR and 95% CI	P-value	OR and 95% CI	P-value	I^2 (%)	Cochran's Q P-value
rs10931936	2	201852173	A	0.3	**1.19**	**1.35E-06**	1.10	0.112	**1.14**	**0.018**	1.09	0.036	**1.21**	**0.005**	**1.17 (1.08, 1.28)**	**2.8E-04**	1.12 (1.05, 1.19)	2.7E-04	**1.18 (1.12, 1.25)**	**1.6E-09**	1.15 (1.10, 1.20)	3.3E-09	1.15 (1.09, 1.21)	2.7E-08	8.5	0.35
rs1035142	2	201861323	A	0.38	1.18	4.25E-07	**1.12**	**0.043**	**1.04**	**0.614**	1.07	0.076	**1.16**	**0.0029**	**1.13 (1.05, 1.20)**	**5.7E-05**	1.11 (1.05, 1.17)	1.3E-04	-	-	1.14 (1.09, 1.19)	5.4E-10	1.14 (1.09, 1.19)	6.5E-08	19.3	0.29
rs700635	2	201861470	G	0.28	1.19	1.26E-06	**1.11**	**0.095**	**1.01**	**0.876**	1.09	0.035	**1.22**	**0.003**	**1.11 (1.00, 1.23)**	**5.2E-02**	1.12 (1.06, 1.20)	1.9E-04	-	-	1.15 (1.10, 1.21)	2.4E-09	1.15 (1.10, 1.21)	1.8E-08	8.0	0.50
rs13016963	2	201871056	A	0.37	**1.18**	**5.68E-07**	1.129	0.035	**1.11**	**0.045**	1.07	0.077	**1.17**	**0.0019**	**1.14 (1.06, 1.23)**	**2.8E-04**	1.11 (1.06, 1.18)	9.2E-05	**1.16 (1.11, 1.22)**	**1.3E-09**	1.14 (1.09, 1.19)	8.6E-10	1.14 (1.09, 1.19)	6.8E-08	18.7	0.30
rs10932444	2	213292933	C	0.23	**1.17**	**5.19E-05**	**1.05**	**0.479**	**1.02**	**0.628**	1.01	0.780	-	-	-	-	-	-	-	-	-	-	-	-	-	
rs11604821	11	69061318	G	0.36	**1.17**	**4.23E-06**	1.130	0.036	**1.04**	**0.478**	1.03	0.424	**1.06**	**0.264**	**1.05 (0.98, 1.13)**	**1.9E-01**	1.06 (1.01, 1.13)	2.9E-02	**1.11 (1.06, 1.17)**	**2.0E-05**	1.10 (1.06, 1.15)	3.8E-06	1.10 (1.03, 1.17)	3.1E-03	50.7	0.11
rs1485993	11	69071595	A	0.37	**1.19**	**4.15E-07**	1.096	0.106	**1.07**	**0.188**	1.05	0.243	**1.08**	**0.143**	**1.08 (1.00, 1.16)**	**4.9E-02**	1.07 (1.01, 1.13)	1.7E-02	**1.13 (1.08, 1.19)**	**5.0E-07**	1.12 (1.07, 1.16)	4.6E-07	1.11 (1.04, 1.18)	1.2E-03	48.9	0.12
rs497356	11	69076356	A	0.37	1.19	3.84E-07	**1.09**	**0.149**	**0.95**	**0.540**	1.05	0.241	-	-	-	-	-	-	-	-	-	-	-	-	-	
rs11263498	11	69091948	T	0.37	1.19	3.24E-07	**1.08**	**0.171**	**1.03**	**0.692**	1.06	0.192	**1.10**	**0.076**	**1.08 (1.01, 1.15)**	**2.8E-02**	1.08 (1.02, 1.14)	1.1E-02	-	-	1.12 (1.07, 1.17)	1.7E-07	1.11 (1.05, 1.18)	4.6E-04	45.0	0.14
rs1801516	11	107680672	A	0.13	**0.79**	**4.80E-07**	**0.81**	**0.010**	**0.88**	**0.019**	0.87	0.014	**0.92**	**0.236**	**0.88 (0.81, 0.94)**	**4.9E-04**	0.87 (0.81, 0.94)	3.4E-04	**0.84 (0.79, 0.91)**	**4.7E-06**	0.84 (0.79, 0.89)	3.4E-09	0.84 (0.78, 0.90)	1.7E-06	28.3	0.24
rs7139314	12	109408254	A	0.10	**1.24**	**5.76E-05**	**1.01**	**0.960**	**1.03**	**0.635**	1.03	0.680	-	-	-	-	-	-	-	-	-	-	-	-	-	
rs9515125	13	109346424	G	0.46	**0.81**	**4.85E-06**	**0.94**	**0.282**	**1.01**	**0.894**	1.00	0.938	-	-	-	-	-	-	-	-	-	-	-	-	-	
rs45430	21	41667951	G	0.39	**0.85**	**5.60E-07**	**0.91**	**0.091**	**0.90**	**0.008**	0.91	0.013	**0.91**	**0.064**	**0.91 (0.86, 0.96)**	**2.4E-04**	0.91 (0.86, 0.96)	4.2E-04	**0.88 (0.85, 0.92)**	**1.5E-09**	0.88 (0.85, 0.92)	2.9E-09	0.88 (0.85, 0.92)	2.9E-09	0.0	0.41

Supplementary Note

Samples

The data analysed here consist of a combination of Phase 1 (previously published [2]) and Phase 2 of a GWA study of melanoma cases and controls, contributed by GenoMEL participating groups. Groups were asked to prioritise samples from melanoma cases with a family history (but confirmed as not having a germline *CDKN2A* mutation), multiple primaries or onset before age 40 years in order to "enrich" the case series for genetic susceptibility, thereby increasing power to identify germline variation affecting risk [8]. Family history was restricted to 3 cases within the family to reduce the risk of including individuals with a high-penetrance mutation. Furthermore, persons with germline *CDKN2A* mutations were excluded independently of their family history. Controls were recruited from the same populations as the cases by the same research groups.

Genotyping was conducted in two phases. The Phase 1 genotyping of GenoMEL samples was conducted through ServiceXS in Leiden, The Netherlands, using the Illumina HumanHap300 BeadChip version 2 duo array (with 317k tagging SNPs), with the exception of the French samples (cases genotyped by Centre National de Génotypage (CNG) in Paris using the Illumina Humancnv370k array and controls genotyped by CNG on the Illumina HumanHap300 Beadchip version 2). Similarly, the majority of the GenoMEL Phase 2 samples were genotyped by ServiceXS on the Illumina 610k array, with the exception once more of the French controls (genotyped by CNG on the Illumina 610k array). Both Phases 1 and 2 were supplemented by UK controls from the WTCCC[9]; these were genotyped on the Illumina HumanHap 1.2 million array, but any SNPs not on the 610k array were discarded. 1,333 controls from the WTCCC (1958 cohort) were used in Phase 1, leaving 4,249 WTCCC controls (1958 cohort and blood donors) for inclusion in Phase 2. Given that we had far more UK controls than cases in which to replicate any GWA findings (see below) we excluded some of the WTCCC controls to supplement the replication series. We already held DNA on 1,344 of the WTCCC blood donor controls (allowing us to genotype SNPs not on any array), and power calculations indicated that this split in data was close to optimal. Thus we used 2,905 WTCCC controls (1,512 from the 1958 cohort and 1,393 blood donors) in Phase 2, retaining 1,344 blood donors for replication.

We defined the research groups by their geographical locations, but to enhance power identified regions within which the data from individual groups could be pooled. These regions were: Scandinavia (Lund, Stockholm and Norway), Italy (Genoa and Emilia-Romagna), UK/Netherlands (Leeds, Leiden and Glasgow), France, Spain, Israel and Poland. The Australian samples previously included in Phase 1[2] were excluded from this analysis, as many of them are used in the Australian replication GWA set.

Quality Control (QC) Methods

Genotypes were called using the proprietary software supplied by Illumina (BeadStudio, version 3.2), with imported cluster centers based on HapMap samples (supplied by Illumina) and call threshold set at 0.15 as recommended by Illumina. Some problems with poor chip quality were identified, and where possible samples with low (<97%) call rates were re-genotyped.

Sample exclusions

Samples were excluded for any of the following reasons: (a) a call rate of less than 97% (of the total number of SNPs on the chip); (b) evidence of non-European origin from PCA (see PCA and Population Stratification in Online Methods); (c) sex as ascertained by genotyping not matching reported sex; (d) evidence of first degree relationship or identity with another sample in either Phase 1 or 2; (e) recommendation to be excluded by the WTCCC. Sex was investigated by calculating the heterozygosity rate on the X-chromosome markers within Beadstudio; persons with > 10% heterozygosity were classified as female. Relationship analysis was carried out in PLINK[10] using estimated identity-by-descent sharing: when two persons were at least as related as first degree relatives, one of the samples was excluded.

SNP QC

SNPs may be poorly genotyped on one platform but not on another. Similarly SNPs may be poorly genotyped as a result of sample handling. Thus we applied QC to SNPs within each platform and genotyping center, giving five sets of data:

(i) GenoMEL samples genotyped by ServiceXS on the Illumina 317k array, (ii) GenoMEL samples genotyped by ServiceXS on the Illumina 610k array, (iii) French controls genotyped by CNG on the Illumina 610k array, (iv) French samples genotyped by CNG on the on the Illumina 317k and 370k arrays, (v) UK controls genotyped by the Wellcome Trust Sanger Institute on the Illumina 1.2M array. Within each of these cohorts we excluded SNPs for one of two reasons: (a) HWE p-value<10^{-20} in controls, or (b) callrate < 97%, or (for the final data set (v) only) (c) recommendation for exclusion by the WTCCC. SNPs could therefore be excluded from just a subset of our entire sample.

When these data were combined, some SNPs that passed QC but were non-polymorphic in one or more of the datasets differed greatly in frequency across datasets, a feature that seems to have arisen through genotype software mis-specifying the allele for monomorphic SNPs in some centers. Thus, when combining data we further excluded any SNP that differed in frequency between groups by >0.8. This resulted in a final analysable dataset of 594,997 SNPs.

When interpreting results we also took into account the concordance of results with neighbouring SNPs and the minor allele frequency (MAF) of the SNP.

QC results

Sample exclusions

Samples were excluded for reasons of either (a) low callrate (431 samples), (b) non-European ethnicity (131 samples), (c) genotyped sex not matching recorded sex (27 samples), (d) relatedness to another sample (66 samples) or (e) recommendation for exclusion by WTCCC (349 samples). This resulted in the exclusion of 330 (6.9%) GenoMEL samples, 314 (11.4%) samples genotyped at CNG and 360 (8.5%) WTCCC samples.

SNP QC

SNPs were excluded as follows: (i) GenoMEL 317k samples (7,176 SNPs excluded - 2.3%), (ii) GenoMEL 610k samples (38,063 SNPs excluded - 6.1%), (iii) French 610k controls (49,489 SNPs excluded - 8.0%), (iv) French 370k samples (51,349 SNPs excluded - 13.9%), (v) WTCCC NBS samples (27,052 SNPs excluded - 4.5%) and (vi) WTCCC 1958 cohort samples (28,563 SNPs excluded - 4.7%). A further 931 SNPs were excluded because they varied greatly in frequency between cohorts (see above).

For the key SNPs in the four loci showing some evidence of replication we examined the cluster plots from BeadStudio (Supplementary Figure 5) separately for the Phase 1 GenoMEL data (317k array) and Phase2 data (610k array), showing clearly defined clusters. In addition approximately 1000 samples from the GWA analysis were also genotyped using Taqman (as for the replication genotyping). The genotyping showed good concordance: RS13016963 100% agreement from 1004 samples, RS1485993 one sample discordant from 982, RS1801516 100% agreement from 1002, RS45430 5 samples discordant from 940.

Quantile-quantile plot and adjusted analyses

We produced quantile-quantile plots, using the results of the trend test. Estimates of over-dispersion were λ=1.48 for the unstratified analysis, dropping to λ=1.27 when we exclude the Israeli samples. Our final analysis, stratifying by geographic region gives λ=1.06. These results suggest that there was some stratification in our sample but that this is adequately corrected for by incorporating regional information. Further adjustment, for finer scale geographic region or by including principal components brings no improvement in λ.

For the key SNPs in loci showing some evidence of replication the following p-values were attained when performing i) a trend test stratified by region and phase: rs13016963 5.1×10^{-7}, rs45430 5.5×10^{-7}, rs1801516 4.8×10^{-7}, rs1485993 4.2×10^{-7}, ii) a trend test stratified by region excluding Polish and Israeli samples: rs13016963 1.3×10^{-5}, rs45430 1.7×10^{-6}, rs1801516 3.8×10^{-7}, rs1485993 1.4×10^{-6}, iii) a logistic regression adjusted for region and the first four PCs: rs13016963 3.4×10^{-6}, rs45430 1.9×10^{-7}, rs1801516 6.0×10^{-7}, rs1485993 3.4×10^{-7}.

Details of Supplementary Table 2

Supplementary Table 2 shows the evidence for previously identified melanoma loci as found in this study. The SNPs reported for each locus are either those which were identified in the original study or the strongest hits in this study.

Phase 1 of this study[2] reported genome-wide significance for three of these loci (*CDKN2A/MTAP*, *TYR* and *MC1R*). About half of the samples used in our current study come from Phase 1 (see Supplementary Table 1)

The SNP rs401681 which is in the 5' region of *TERT* and close to *CLMPT1L* was reported by Rafnar et al.[6]; estimated effect sizes from that study are similar to those found here.

SLC45A2 has been examined by a number of groups; the only SNPs on the Illumina 610k array which have also been examined previously are: rs26722[11,12] (p=0.0003 for this study), rs35401[13] (p=0.13) and rs35414[13] (p=0.06).

IRF4 is a known pigmentation gene[14]. The two SNPs listed have both been reported as being associated with melanoma, although the effect of *IRF4* on melanoma risk may be site-specific, with the strongest effect observed for truncal melanoma[4]. Our results show an effect in the same direction as that previously observed, but it is weaker and the lack of significance here may thus be due to low power.

The top SNPs for the 9p21 region containing *CDKN2A* and *MTAP* were both identified in genome-wide studies of melanoma[2] and nevi[5]; the top SNP for the chromosome 22 region adjacent to *PLA2G6* was identified in the genome-wide study of nevi[5] and replicated for melanoma[2].

The top SNP on chromosome 16 in our previous genome-wide study of melanoma, rs258322[2], is distant from *MC1R*, the pigmentation gene associated with red hair and skin sensitivity to the sun, but analysis of *MC1R* variants in the same population showed that the signal from rs258322 is explained by multiple rare *MC1R* variants, a number of which were red hair variants [15]. Similarly rs1393350, the top SNP on chromosome 11 in the *TYR* region, another pigmentation gene, was shown to be explained by a coding variant of *TYR*[2].

Finally, a genome-wide study using pooled DNA samples[3], identified a locus on chromosome 20 in the vicinity of *ASIP*. The two most significant SNPs here were rs910873 and rs1885120. However, a haplotype in the vicinity of *ASIP* involving rs1015362 and rs4911414 were found to be associated with pigmentation and melanoma risk; these may well be same signal but this remains to be proven[13,16].

Imputation

Imputation of ungenotyped SNPs was conducted using IMPUTEv2[17,18], which predicts the genotypes of unobserved SNPs by means of a hidden Markov model using the genotype data at observed markers and a set of known haplotypes (in this case European samples from HapMap release 2 (Feb 2009) and the 1000 Genomes pilot data (Mar 2010)). As the method is quite computationally intensive, it was applied only to those regions in which at least one SNP reached a p-value $<10^{-5}$ in the stratified CA trend test. We imputed 0.5Mb either side of any SNP that reached the required p-value, as well as a 250kb buffer either side to avoid end effects. More stringent QC was applied to genotyped SNPs for this analysis, excluding any with HWE p-value in controls $<10^{-4}$ or MAF<0.03. We assumed an effective population size of 11,400 and analysed the results by applying SNPTEST2 [9,17] to the expected genotype counts assuming an additive mode of inheritance. All four imputed SNPs that were followed up for replication had maximum posterior probability of at least 0.9 in at least 97% of samples, suggesting they were well-imputed. Furthermore almost 1,000 of the Leeds GenoMEL samples were independently genotyped at three of these four SNPs (rs1035142, rs11263498 and rs700635).

The most likely imputed genotype and the directly genotyped samples were discrepant in only 0.3% of comparisons (6/999, 2/971 and 2/997 samples respectively).

Replication

Replication samples

We sought to replicate our findings with two GWA datasets from Australia and Houston and further samples consisting of case-control series from Leeds, Cambridge and the Netherlands, as well as UK controls from WTCCC:

(i) Australia

926 cases and 3,956 controls were genotyped on the Illumina HumanHap610k array and 1,242 cases and 431 controls genotyped on the Illumina Human1M-OMNI array as part of a separate GWA study of melanoma ([19]). Data were analysed by regressing case-control status on genotype (coded according to an additive model) adjusting for the first 10 PCs. Imputed SNPs were estimated using MACH2[20,21].

(ii) Houston

1,804 cases and 1,026 controls were genotyped on the Illumina Human1M-OMNI array. Data were analysed by regressing case-control status on genotype (coded according to an additive model) adjusting

for the first 2 PCs (Amos et al., in preparation). SNPs not included on the arrays were imputed using MACH [20,21].

(iii) Leeds case-control study

The Leeds-based case-control study recruited 1,897 population-based incident melanoma cases diagnosed between September 2000 and December 2006 from a geographically defined area of Yorkshire and the Northern region of the UK (63% response rate)[2,5,22,23]. Cases were identified by clinicians, pathology registers and via the Northern and Yorkshire Cancer Registry and Information Service to ensure overall ascertainment. For all but 18 months of the study period, recruitment was restricted to patients with Breslow thickness of at least 0.75mm. Controls were ascertained by contacting general practitioners to identify eligible individuals. These controls were frequency-matched with cases for age and sex from general practitioners who had also had cases as part of their patient register. Overall there was a 55% response rate for controls (513 subjects).

The first 960 of the cases recruited and all controls were examined by trained interviewers who performed a standardised examination of the skin, recording nevi by anatomical site and size.

647 of the cases and 413 of the controls from this study were genotyped genome-wide in either Phase 1 (with the 317k array) or Phase 2 (the 610k array). The 937 cases who were not genotyped genome-wide were genotyped in the replication phase of this study, as were 100 controls.

(iv) Cambridge case-control study

383 cases and 378 controls recruited by the University of Cambridge were genotyped in the replication series. The cases and controls were recruited as part of the SEARCH study[24,25], an ongoing population-based study in Eastern England. Cases were ascertained through the Eastern Cancer Registry and Information Centre, and were aged between 18 and 70 years at diagnosis. Controls were drawn from SEARCH and EPIC-Norfolk. Details of these studies have been previously published[24,25].

(v) Replication samples from Leiden, Netherlands

The Dutch case-control cohort consists of in total 259 consented melanoma patients and 214 friend or spouse controls. The cohort was recruited in several hospitals in the Netherlands for which local ethical approval was obtained.

(vi) WTCCC UK Blood Service Control Group

1,344 controls from the UK Blood Service Control Group genotyped as part of the WTCCC[9] were also included in the replication series.

Replication Genotyping

The Leeds, Cambridge and Leiden samples in the replication phase of this study were genotyped for SNPs of interest using Taqman SNP genotyping assays (Applied Biosystems, Foster City, USA). The SNPs rs10931936, rs1035142, rs700635, rs13016963 (*CASP8*); rs11604821, rs1485993, rs11263498 (*CCND1*); rs1801516 (*ATM*); and rs45430 (*MX2*) were genotyped using the Taqman assays C___2960444_10, C___8823871_10, C___8823870_1_, C__30787149_10, C___3033904_10, C___8762595_10, C___3033901_10, C__26487857_10, and C___2564407_10 respectively (Applied Biosystems). 2ul PCR reactions were performed in 384 well plates using 10ng of DNA (dried), using 0.05 ul assay mix and 1ul Universal Master Mix (Applied Biosystems) according to the manufacturers' instructions. End point reading of the genotypes was performed using an ABI 7900HT Real-time PCR system (Applied Biosystems).WTCCC samples were genotyped on the Illumina Human1M-OMNI array and, where the SNP was not present on this array, by direct genotyping as above.

Replication Analysis

For each region, the SNP chosen as the primary SNP for replication was the most significant genotyped SNP that is on both the Illumina Human1M-OMNI array and the HumanHap610 array (as replication samples were genotyped on both arrays). If no such SNP existed in the region with $p<10^{-4}$, the two most significant genotyped SNPs were followed up, as were the two most significant imputed SNPs on the Human1M-OMNI array. All SNPs chosen for replication were investigated in the two GWA datsets. Those showing evidence of replication were further genotyped in the case-control datasets from the UK and Netherlands. The UK and Netherlands data were analysed by regressing case-control status on genotype (coded according to an additive model) combining the UK cases and controls (Leeds and Cambridge cases, Leeds, Cambridge and WTCCC controls) into one "UK" series and performing a stratified analysis with the Leiden case-control samples. As an additional QC measure we checked that there were no significant differences in frequency for any of the 9 SNPs followed up for replication, between genotyping centers within the same country (UK and France). For the 18 tests, the lowest p-value was 0.03 and frequencies never differed between genotyping centers by more than 0.04, suggesting little difference between either the samples or the genotyping quality at the different centers for these SNPs.

CCND1 locus

The *CCND1* locus on chromosome 11 showed some evidence of replication (replication OR=1.07 (1.01, 1.13), p=0.017 for rs1485993, the most-strongly associated genotyped SNP from the GWA study, Table 1), although this was not significant after adjusting for multiple testing. We have used imputation to examine the evidence for association in the region in the GenoMEL GWA data (Supplementary Figure 4). The associated SNPs are in a region of low LD, and genotyped or imputed SNPs showing association at $p<10^{-5}$ are within a 55kb region within the *CCND1* gene. The estimated effects show no evidence of heterogeneity by region, with all centers in the genome-wide and replication samples apart from Scandinavia showing a

per-allele OR above 1 (Figure S4). From analysis of the Leeds case-control study, there is no evidence of an effect of this SNP on either nevus count (0.01% of variance explained, p=0.83) or pigmentation phenotype (0.02% of variance explained, p=0.55). A genome-wide study of hair-color found a replicable hit in the region of *CCND1* with rs3750965, although this is about 500kb away and shows no significance in our study[26]. More interestingly a genome-wide study of breast cancer found a hit (rs614367) within 60kb of our top hit which is nominally significant in our study (p=0.017)[27].

Power of the GWA study

With the current sample size of the GWA discovery study, the power is good (>80%) to detect a SNP with a genotype relative risk (GRR) above 1.3 and MAF>0.08 or GRR>1.2 and MAF>0.2 at a significance level of 10^{-5}, while power to detect a GRR of 1.1 is never greater than 12%. For the four new regions of interest, using effect size estimates from the genome-wide data, powers are 86%, 79%, 73%, and 91% for *CASP8, ATM*, *MX2* and *CCND1* respectively. However, if instead we use the estimates of effect size from the replication studies, powers are 17%, 7%, 9% and 1% for *CASP8*, *ATM*, *MX2* and *CCND1* respectively. The estimates from the GWA study are subject to the expected inflation in effect sizes caused by the so-called 'winner's curse' [28]; the estimates from the replication studies are not subject to this bias. It should also be noted that none of the estimates are derived from a representative set of incident cases, whereas GenoMEL cases are genetically enriched, which may also increase the effect size. The low power to detect a GRR of 1.1 suggests that there may be many other genetic regions with a similar effect on melanoma risk, which we are currently underpowered to detect.

The power to reach a genome-wide significance level of 5×10^{-8} using the combined discovery and replication data is 80% for a SNP with an OR of 1.2, but for an OR of 1.1 the maximum power attainable is 0.41 (when MAF=0.5); for an OR of 1.07 the maximum power possible is 0.04 (when MAF=0.5)

GenoMEL Collaboration

Australian Melanoma Family Study: Graham J. Mann, John L. Hopper, Joanne F. Aitken, Bruce K. Armstrong, Graham G. Giles, Richard F. Kefford, Anne Cust, Mark Jenkins, Helen Schmid.

Barcelona: The participants of GenoMEL in Barcelona: Paula Aguilera, Celia Badenas, Cristina Carrera, Francisco Cuellar, Daniel Gabriel, Estefania Martinez, Melinda Gonzalez, Pablo Iglesias, Josep Malvehy, Rosa Marti-Laborda, Montse Mila, Zighe Ogbah, Joan-Anton Puig Butille, Susana Puig and Other members of the Melanoma Unit: Llúcia Alós, Ana Arance, Pedro Arguís, Antonio Campo, Teresa Castel, Carlos Conill, Jose Palou, Ramon Rull, Marcelo Sánchez, Sergi Vidal-Sicart, Antonio Vilalta, Ramon Vilella.

Brisbane: The Queensland study of Melanoma: Environmental and Genetic Associations (Q-MEGA) Principal Investigators are: Nicholas G. Martin, Grant W. Montgomery, David Duffy, David Whiteman, Stuart MacGregor, Nicholas K. Hayward. **The Australian Cancer Study (ACS) Principal Investigators are:** David Whiteman, Penny Webb, Adele Green, Peter Parsons, David Purdie, Nicholas Hayward.

Emilia-Romagna: Maria Teresa Landi, Donato Calista, Giorgio Landi, Paola Minghetti, Fabio Arcangeli, Pier Alberto Bertazzi

Genoa: Department of Internal Medicine (DIMI), University of Genoa: Giovanna Bianchi-Scarra, Paola Ghiorzo, Lorenza Pastorino, William Bruno, Linda Battistuzzi, Sara Gargiulo, Sabina Nasti, Sara Gliori, Paola Origone, Virginia Andreotti; Medical Oncology Unit, National Institute for Cancer Research: Paola Queirolo.

Glasgow: Rona Mackie, Julie Lang

Leeds: Julia A Newton Bishop, Paul Affleck, Jennifer H Barrett, D Timothy Bishop, Jane Harrison, Mark M Iles, Juliette Randerson-Moor, Mark Harland, John C Taylor, Linda Whittaker, Kairen Kukalizch, Susan Leake, Birute Karpavicius, Sue Haynes, Tricia Mack, May Chan, Yvonne Taylor, John Davies, Paul King.

Leiden: Department of Dermatology, Leiden University Medical Centre: Nelleke A Gruis, Frans A van Nieuwpoort, Coby Out, Clasine van der Drift, Wilma Bergman, Nicole Kukutsch, Jan Nico Bouwes Bavinck. Department of Clinical Genetics, Centre of Human and Clinical Genetics, Leiden University Medical Centre: Bert Bakker, Nienke van der Stoep, Jeanet ter Huurne. Department of Dermatology, HAGA Hospital, The Hague: Han van der Rhee. Department of Dermatology, Reinier de Graaf Groep, Delft: Marcel Bekkenk. Department of Dermatology, Sint Franciscus Gasthuis, Rotterdam: Dyon Snels, Marinus van Praag. Department of Dermatology, Ghent University Hospital, Ghent, Belgium: Lieve Brochez & colleagues. Department of Dermatology, St. Radboud University Medical Centre, Nijmegen: Rianne Gerritsen & colleagues. Department of Dermatology, Rijnland Hospital, Leiderdorp: Marianne Crijns & colleagues. Dutch patient organisation, Stichting Melanoom, Purmerend. The Netherlands Foundation for the detection of Hereditary Tumors, Leiden: Hans Vasen.

Lund: Lund Melanoma Study Group: Håkan Olsson, Christian Ingvar, Göran Jönsson, Åke Borg, Anna Måsbäck, Lotta Lundgren, Katja Baeckenhorn, Kari Nielsen, Anita Schmidt Casslén.

Norway: Oslo University Hospital: Per Helsing, Per Arne Andresen, Helge Rootwelt. **University of Bergen:** Lars A. Akslen, Anders Molven.

Paris: Marie-Françoise Avril, Brigitte Bressac-de Paillerets, Valérie Chaudru, Nicolas Chateigner , Eve Corda, Patricia Jeannin, Fabienne Lesueur, Mahaut de Lichy, Eve Maubec, Hamida Mohamdi, Florence Demenais and the French Family Study Group including the following Oncogeneticists and Dermatologists: Pascale Andry-Benzaquen, Bertrand Bachollet, Frédéric Bérard, Pascaline Berthet, Françoise Boitier, Valérie Bonadona, Jean-Louis Bonafé, Jean-Marie Bonnetblanc, Frédéric Cambazard, Olivier Caron, Frédéric Caux, Jacqueline Chevrant-Breton, Agnès Chompret (deceased), Stéphane Dalle, Liliane Demange, Olivier Dereure, Martin-Xavier Doré, Marie-Sylvie Doutre, Catherine Dugast, Laurence Faivre, Florent Grange, Philippe Humbert, Pascal Joly, Delphine Kerob, Christine Lasset, Marie Thérèse Leccia, Gilbert Lenoir, Dominique Leroux, Julien Levang, Dan Lipsker, Sandrine Mansard, Ludovic Martin, Tanguy Martin-Denavit, Christine Mateus, Jean-Loïc Michel, Patrice Morel, Laurence Olivier-Faivre, Jean-Luc Perrot, Caroline Robert, Sandra Ronger-Savle, Bruno Sassolas, Pierre Souteyrand, Dominique Stoppa-Lyonnet, Luc Thomas, Pierre Vabres, Eva Wierzbicka.

Philadelphia: David Elder, Peter Kanetsky, Jillian Knorr, Michael Ming, Nandita Mitra, Althea Ruffin, Patricia Van Belle

Poland: Tadeusz Dębniak, Jan Lubiński, Aneta Mirecka, Sławomir Ertmański

Slovenia: Srdjan Novakovic, Marko Hocevar, Barbara Peric, Petra Cerkovnik

Stockholm: Veronica Höiom, Johan Hansson

Sydney: Graham J. Mann, Richard F. Kefford, Helen Schmid, Elizabeth A. Holland.

Tel Aviv: Esther Azizi, Gilli Galore-Haskel, Eitan Friedman, Orna Baron-Epel, Alon Scope, Felix Pavlotsky, Emanuel Yakobson, Irit Cohen-Manheim, Yael Laitman, Roni Milgrom, Iris Shimoni, Evgeniya Kozlovaa

See also: www.genomel.org

Other Collaboration

Cambridge: Alison Dunning, Doug Easton, Liz Margerison, Karen Pooley, Phillip Smith.

References

1. Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263-5 (2005).
2. Bishop, D.T. *et al.* Genome-wide association study identifies three loci associated with melanoma risk. *Nat Genet* **41**, 920-5 (2009).
3. Brown, K.M. *et al.* Common sequence variants on 20q11.22 confer melanoma susceptibility. *Nat Genet* **40**, 838-40 (2008).
4. Duffy, D.L. *et al.* IRF4 variants have age-specific effects on nevus count and predispose to melanoma. *Am J Hum Genet* **87**, 6-16 (2010).
5. Falchi, M. *et al.* Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. *Nat Genet* **41**, 915-9 (2009).
6. Rafnar, T. *et al.* Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. *Nat Genet* **41**, 221-7 (2009).
7. Turnbull, C. *et al.* Variants near DMRT1, TERT and ATF7IP are associated with testicular germ cell cancer. *Nature Genetics* **42**, 604-U178 (2010).
8. Antoniou, A.C. & Easton, D.F. Polygenic inheritance of breast cancer: Implications for design of association studies. *Genet Epidemiol* **25**, 190-202 (2003).
9. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661-78 (2007).
10. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559-75 (2007).
11. Guedj, M. *et al.* Variants of the MATP/SLC45A2 gene are protective for melanoma in the French population. *Hum Mutat* **29**, 1154-60 (2008).
12. Fernandez, L.P. *et al.* SLC45A2: a novel malignant melanoma-associated gene. *Hum Mutat* **29**, 1161-7 (2008).
13. Nan, H., Kraft, P., Hunter, D.J. & Han, J. Genetic variants in pigmentation genes, pigmentary phenotypes, and risk of skin cancer in Caucasians. *Int J Cancer* **125**, 909-17 (2009).
14. Sturm, R.A. Molecular genetics of human pigmentation diversity. *Hum Mol Genet* **18**, R9-17 (2009).
15. Demenais, F. *et al.* Importance of sequencing rare variants after a genome-wide association study (GWAS): the MC1R gene, 16q24 region and melanoma story. in *American Society of Human Genetics* (2009).
16. Gudbjartsson, D.F. *et al.* ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. *Nat Genet* **40**, 886-91 (2008).
17. Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet* **39**, 906-13 (2007).
18. Howie, B.N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* **5**, e1000529 (2009).
19. Macgregor, S. *et al.* Genome-wide association study identifies a new melanoma susceptibility locus at 1q21.3. *Nature Genetics* **In Press**(2011).
20. Li, Y., Willer, C., Sanna, S. & Abecasis, G. Genotype imputation. *Annu Rev Genomics Hum Genet* **10**, 387-406 (2009).
21. Li, Y., Willer, C.J., Ding, J., Scheet, P. & Abecasis, G.R. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* **34**, 816-34 (2010).
22. Newton-Bishop, J.A. *et al.* Relationship between sun exposure and melanoma risk for tumours in different body sites in a large case-control study in a temperate climate. *Eur J Cancer* (2010).
23. Newton-Bishop, J.A. *et al.* Melanocytic nevi, nevus genes, and melanoma risk in a large case-control study in the United Kingdom. *Cancer Epidemiol Biomarkers Prev* **19**, 2043-54 (2010).
24. Pooley, K.A. *et al.* Common single-nucleotide polymorphisms in DNA double-strand break repair genes and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **17**, 3482-9 (2008).

25. Pooley, K.A. *et al.* No association between TERT-CLPTM1L single nucleotide polymorphism rs401681 and mean telomere length or cancer risk. *Cancer Epidemiol Biomarkers Prev* **19**, 1862-5 (2010).
26. Eriksson, N. *et al.* Web-based, participant-driven studies yield novel genetic associations for common traits. *PLoS Genet* **6**, e1000993 (2010).
27. Turnbull, C. *et al.* Genome-wide association study identifies five new breast cancer susceptibility loci. *Nat Genet* **42**, 504-7 (2010).
28. Lohmueller, K.E., Pearce, C.L., Pike, M., Lander, E.S. & Hirschhorn, J.N. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* **33**, 177-82 (2003).