Genome-wide association study identifies three loci associated with melanoma risk

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Supplementary Note

Quality control (QC) results

Call rate

We excluded 420 individuals (79.2% of the overall exclusions) whose single nucleotide polymorphism (SNP) call rate was below 97% for the array on which the sample was genotyped. These individuals predominantly came from the French controls (253 individuals – 60.2%).

Non-European Ancestry

In total 64 (1%) individuals (12.1% of the overall exclusions) were removed from subsequent analyses on the basis of apparent non-European ancestry (35.9% or 23 individuals were from the French controls), with a number of these subsequently confirmed with research records.

Sex and relatedness

Sex as inferred from genotyping did not matching reported sex in 18 individuals (3.4% of the overall exclusions). 28 samples (5.3% of the overall exclusions) were removed either because they were identical to or estimated to be first-degree relatives of other samples. The majority of these were case-control pairs and the control samples were removed from further analysis.

Overall QC

In total, 530 (8.9%) samples failed one of these QC criteria (more than half of them from the French control set) and were excluded from subsequent analyses. Of the GenoMEL samples, 1539 cases and 979 GenoMEL controls passed all QC criteria; these were all genotyped at the Service XS laboratory in Leiden (mean call rate 99.6%) apart from 537 cases genotyped at Centre National de Génotypage (CNG) in Paris (mean call rate 99.1%). Of the other control sets, 1543 French controls and 1395 UK controls passed QC, giving a total study size of 1539 cases and 3917 controls with a mean call rate of 98.7% (Supplementary Table 1). Of the cases 1476 (96%) met the criteria defined to enhance for genetic risk.

Falchi et al GWA study of nevus count variation

In an accompanying article Falchi et al[1] report a genome-wide association study of nevus count variation. As described in the introduction to this report, nevi are a major risk factor for melanoma; in particular individuals with increased numbers of nevi are at increased risk of developing melanoma. The Falchi et al study reports an analysis of unselected UK twins whose nevus count has been determined by trained examiners. Their genome-wide study identifies two regions containing genes associated with variation in nevus number which are then replicated in an Australian population. One of these regions on chromosome 9 overlaps with that determined in this study.

The combination of the two studies suggest that the genetic regions on chromosomes 9 and 22 are associated with nevus count variation and this variation influences the risk of melanoma. To examine this hypothesis further requires studies which combine participant information on melanoma and nevus count. For this reason the Leeds case-control study was examined to test the support for this hypothesis. The analysis therefore examines the case-control study simply looking at SNPs in the chromosome 9 region with and without adjustment for nevus count. The findings support the speculation that the genetic region predominantly influences melanoma through nevus count variation.

In terms of samples there are differences in the numbers of cases and controls reported for GenoMEL study as compared to Falchi et al. The discrepancy arises because for the analysis of melanoma risk in the Falchi et al analysis the Leeds study is regarded as testing the hypothesis of the role of nevi; this analysis is based on all cases and controls. In the GenoMEL study where the same case-control study is used to replicate the risk of melanoma the replication can only include those individuals who did not contribute to the genome-wide phase of the analysis. Thus there are fewer individuals recorded in the GenoMEL section of the Leeds case-control study than in the Falchi et al analysis.

QQ plot

We produced quantile-quantile plots, using the results of the trend test for 287794 SNPs with a callrate of at least 97% and excluding some with a very low minor allele frequency. Estimates of over-dispersion [2] were λ=1.14 for the unstratified analysis, λ=1.06 for the analysis stratified by region, and λ=1.03 for the analysis adjusted for region and the first three principal components (from the Principal Components Analysis (PCA) of the GenoMEL samples). These results suggest that there wasn't a great deal of stratification in our sample and that what stratification there was, was adequately corrected for.

"Genetic enrichment" of replication samples

Replication cases were chosen preferentially to be genetically-enriched, categorised as having either (i) a family history or (ii) multiple primaries (without a family history), or (iii) onset before the age of 40 years (in the absence of a family history or multiple primaries). A few persons from GenoMEL groups were genotyped and subsequently found not to have one of the risk criterion ("No criterion"). The Leeds cases from the case-control study were not selected to satisfy these criteria. 1149 of the cases and 964 of the controls in the replication sample were from GenoMEL and 1163 cases and 903 controls were from the Leeds case-control study. 47% of the GenoMEL replication samples were classified as having a family history, 25% as having multiple primary melanoma, 24% as being early onset cases and 3% fulfilling no genetic enrichment criteria. Of the replication samples from the Leeds case-control study 10% had a family history, 2% multiple primary melanoma, 15% early onset and 72% fulfilled no genetic enrichment criteria.

Genotype specific risk

Genotype-specific risks for the five replicated loci in this study were estimated and little evidence was found for departure from additivity. The genotype specific risks (and 95% confidence intervals) for the top SNPs in our replicated regions on chromsomes 9, 11 and 16 are:

rs7023329 (chromosome 9): 0.89 [0.81, 0.99] for heterozygotes and 0.73 [0.64, 0.82] for homozygotes

rs1393350 (chromosome 11) 1.21 [1.10, 1.32] for heterozygotes and 1.80 [1.54, 2.09] for homozygotes

rs258322 (chromosome 16) 1.70 [1.53, 1.89] for heterozygotes and 2.42 [1.74, 3.37] for homozygotes

For the two regions on chromsomes 20 and 22 we followed up on the basis of the findings of other studies the genotype specific risks and 95% confidence intervals are:

rs1885120 (chromosome 20): 1.75 [1.46, 2.09] for heterozygotes and 1.34 [0.67, 2.68] for homozygotes

rs2284063 (chromosome 22): 0.81 [0.71, 0.93] for heterozygotes and 0.68 [0.55, 0.84] for homozygotes

All of the above estimates are based on the results of the genome-wide study and the replication samples except for rs1885120, which was not genotyped in the genome-wide study.

GenoMEL Collaboration

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Supplementary Table 1. Description of genome-wide samples. In total samples from 1650 cases and 4336 controls were included in the genome-wide analysis. Summary information detailing samples contributed and genotyping laboratories is given for participating GenoMEL groups. Also listed are the numbers of samples genotyped, the numbers excluded after quality control, and the remaining numbers of cases and controls and their mean call rates. The numbers of each genetically-enriched case phenotype (family history, multiple primary melanoma or early onset) are given for those cases passing quality control. The genotyping laboratory is either SXS (ServiceXS, Leiden, The Netherlands), CNG (Centre National de Génotypage, Evry, France), or SAN (Sanger Centre, Cambridge, UK). The mean call rate is the average number of genotyped SNPs across all persons retained in the analysis.

			SAMPLE NUMBERS					CASE CATEGORY			
Group	Country	Genotyping Laboratory	Number of Samples Genotyped	Number Excluded After QC	Number of Cases in Statistical Analysis	Number of Controls in Statistical Analysis	Mean call rate	Family History	Multiple Primary Melanoma	Early onset	No criterion
GenoMEL Groups:											
Brisbane	Australia	SXS	191	19	92	80	99.1%	44 (48%)	28 (30%)	20 (22%)	0 (0%)
Sydney	Australia	SXS	196	15	90	91	99.0%	24 (27%)	3 (3%)	63 (70%)	0 (0%)
Paris	France	SXS	197	24	89	84	99.3%	49 (55%)	28 (31%)	12 (13%)	0 (0%)
Paris	France	CNG	477	18	459	0	99.3%	185 (40%)	220 (48%)	46 (10%)	8 (2%)
Emilia-Romagna	Italy	SXS	200	11	96	93	99.4%	48 (50%)	11 (11%)	37 (39%)	0 (0%)
Genoa	Italy	SXS	198	12	92	94	99.5%	45 (49%)	27 (29%)	20 (22%)	0 (0%)
Leiden	Netherlands	SXS	199	8	96	95	99.3%	49 (51%)	18 (19%)	29 (30%)	0 (0%)
Barcelona	Spain	SXS	199	39	74	86	97.8%	24 (32%)	34 (46%)	16 (22%)	0 (0%)
Lund	Sweden	SXS	200	4	99	97	99.7%	55 (56%)	22 (22%)	21 (21%)	1 (1%)
Stockholm	Sweden	SXS	193	20	81	92	98.6%	42 (52%)	26 (32%)	13 (16%)	0 (0%)
Leeds	UK	SXS	374	14	193	167	98.9%	58 (30%)	26 (13%)	57 (30%)	52 (27%)
Leeds	UK	CNG	91	13	78	0	98.6%	45 (58%)	13 (17%)	18 (23%)	2 (3%)
Total GenoMEL			2715	197	1539	979	99.1%	668 (43%)	456 (30%)	352 (23%)	63 (4%)
Other Control Samples:											
French Controls	France	CNG	1824	281	0	1543	97.6%	NA	NA	NA	NA
WTCCC Controls	UK	SAN	1447	52	0	1395	99.4%	NA	NA	NA	NA
Total Other Control Samples			3271	333	0	2938	99.1%				
SUMMARY											
Total Genotyped			**5986**		**1650**	**4336**					
Total Excluded After QC				**530**	**111**	**419**					
Total in Statistical Analysis (% of Genotyped Samples)					**1539 (93.3%)**	**3917 (90.3%)**	98.7%	668 (43%)	456 (30%)	352 (23%)	63 (4%)

Supplementary Table 2. Detailed genome-wide results. The results of the Cochran-Armitage trend test analysis across the genome showing the results for multiple SNPs with $p < 10^{-5}$ and at least one genotyped (G) or imputed (Imp) SNP with a p-value less than 5×10^{-7}. Results are shown for stratified analysis (by geographical region) and unstratified as well as the "minimum p" (ie the minimum of these two results). The OR is the per-allele odds ratio. The call rate is the proportion of persons genotyped successfully for this SNP. For each SNP, we include the minor allele frequency (MAF), the Hardy-Weinberg equilibrium (HWE) p-value for all genotyped controls and the test of the homogeneity of the minor allele frequency across populations. For the imputated SNPs the call rate is recorded as NA. We also computed measures of homogeneity of ORs across geographical location. These were based on the measures suggested by Higgins and Thompson [3]. Q is Cochran's test for OR homogeneity based on a chi-squared distribution while I^2 describes the percentage of total study variance attributable to heterogeneity. Low values of I^2 are consistent with sampling error.

								MAF by Geographical Region					Association Analysis				Homogeneity of ORs across Geographical Regions	
SNP	Chromosome	Base-pair	Genotyped (G) or Imputed (Imp)	Minor Allele	Major Allele	Call rate	HWE for All Controls Combined	Minimum HWE in Controls Across Geographical Regions	Overall MAF	Minimum MAF across Geographical Regions	Maximum MAF across Geographical Regions	Standard Deviation of Regional MAF	P for Homogeneity of MAF Across Geographical Regions	Minimum P	P for Unstratified Analysis	P for Stratified by Geographical Region Analysis	OR Stratified by Region	Q (p-value)	I^2 [Conf. Int.]
rs6730157	2	135623558	G	G	A	1.00	3.65E-15	2.43E-03	0.40	0.27	0.86	0.23	2.30E-148	4.82E-06	4.82E-06	2.04E-01	1.06 [0.97, 1.16]	7.4 (P=0.19)	33 [0, 73]
rs1561277	2	135808531	G	A	C	1.00	1.23E-13	8.85E-03	0.36	0.25	0.78	0.22	1.17E-131	5.21E-07	5.21E-07	4.80E-02	1.10 [1.00, 1.20]	3.2 (P=0.66)	0 [0, 75]
rs1446585	2	136123949	G	G	A	1.00	1.49E-14	7.42E-03	0.35	0.24	0.78	0.22	2.52E-137	3.27E-06	3.27E-06	1.30E-01	1.07 [0.98, 1.18]	3.1 (P=0.68)	0 [0, 75]
rs2011946	2	136534086	G	C	A	1.00	4.39E-06	2.07E-01	0.36	0.26	0.70	0.18	9.09E-95	6.88E-07	6.88E-07	1.21E-02	1.12 [1.03, 1.23]	1.6 (P=0.90)	0 [0, 75]
rs932206	2	136541742	G	G	A	1.00	4.78E-07	1.64E-01	0.46	0.35	0.83	0.18	1.56E-91	2.68E-07	2.68E-07	3.15E-02	1.10 [1.01, 1.20]	5.5 (P=0.36)	10 [0, 77]
rs10515229	5	95161775	G	G	A	1.00	7.82E-01	6.04E-01	0.10	0.07	0.11	0.01	7.00E-02	6.21E-06	6.57E-06	6.21E-06	1.38 [1.20, 1.60]	2.0 (P=0.85)	0 [0, 75]
rs6894498	5	95165802	Imp	A	G	NA	-	-	-	-	-	-	-	6.31E-08	1.17E-07	6.31E-08	1.50 [1.30, 1.73]	-	-
rs871775	5	95184524	G	A	G	0.99	6.01E-01	1.36E-01	0.12	0.08	0.16	0.02	1.25E-01	1.14E-05	2.32E-05	1.14E-05	1.34 [1.17, 1.52]	6.4 (P=0.27)	22 [0, 66]
rs4636294	9	21737803	G	G	A	1.00	7.49E-01	6.73E-02	0.51	0.48	0.59	0.04	3.83E-03	9.86E-07	9.86E-07	1.77E-05	0.83 [0.76, 0.90]	9.9 (P=0.08)	50 [0, 80]
rs2218220	9	21746089	G	A	G	0.99	5.87E-01	6.73E-02	0.51	0.48	0.59	0.04	2.38E-03	9.03E-07	9.03E-07	1.85E-05	0.83 [0.76, 0.90]	10.2 (P=0.07)	51 [0, 80]
rs1335510	9	21747803	G	C	A	1.00	8.18E-01	4.72E-01	0.41	0.33	0.44	0.04	1.66E-03	5.51E-06	5.51E-06	1.14E-04	0.84 [0.77, 0.92]	8.1 (P=0.15)	39 [0, 76]
rs935053	9	21773922	Imp	A	G	NA	-	-	-	-	-	-	-	2.37E-07	2.37E-07	4.72E-06	0.81 [0.74, 0.89]	-	-
rs10757257	9	21796564	G	A	G	1.00	5.51E-01	2.28E-01	0.41	0.33	0.43	0.04	5.69E-03	5.61E-06	5.61E-06	3.39E-05	0.83 [0.76, 0.91]	8.8 (P=0.12)	43 [0, 78]
rs7023329	9	21806528	G	G	A	1.00	8.48E-01	4.68E-01	0.50	0.48	0.60	0.04	2.47E-03	5.89E-07	5.89E-07	1.14E-05	0.82 [0.75, 0.90]	10.1 (P=0.07)	50 [0, 80]
rs1042602 (A)	11	88551344	G	A	C	1.00	3.30E-01	1.85E-02	0.39	0.33	0.49	0.07	4.91E-10	1.13E-01	2.37E-01	1.13E-01	0.93 [0.85, 1.02]	1.7 (P=0.89)	0 [0, 75]
rs1393350	11	88650694	G	A	G	1.00	5.38E-02	5.66E-02	0.27	0.18	0.29	0.04	5.84E-04	4.28E-08	1.94E-07	4.28E-08	1.30 [1.19, 1.43]	4.3 (P=0.50)	0 [0, 75]
rs1126809 (B)	11	88657609	G	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
rs1847142 (C)	11	88661222	Imp	A	G	NA	-	-	-	-	-	-	-	8.35E-09	4.22E-08	8.35E-09	1.33 [1.21, 1.47]	-	-
rs1806319	11	88677584	G	G	A	0.99	2.21E-01	9.10E-03	0.34	0.30	0.38	0.03	2.55E-03	2.49E-06	2.93E-05	2.49E-06	1.24 [1.13, 1.35]	3.1 (P=0.69)	0 [0, 75]
rs10830253 (D)	11	88667691	G	G	T	-	-	-	-	-	-	-	-				-		
rs2353033	16	87913062	G	G	A	0.99	1.00E+00	2.19E-01	0.41	0.32	0.48	0.07	2.76E-16	3.32E-07	8.72E-06	3.32E-07	1.26 [1.15, 1.38]	5.3 (P=0.38)	5 [0, 76]
rs352935	16	88176081	G	G	A	0.99	7.70E-02	1.06E-01	0.46	0.38	0.54	0.06	2.42E-14	5.78E-06	2.97E-05	5.78E-06	1.22 [1.12, 1.33]	5.2 (P=0.39)	4 [0, 76]
rs164741	16	88219799	G	A	G	0.97	4.64E-01	8.00E-02	0.29	0.19	0.37	0.07	4.15E-17	1.50E-06	1.33E-05	1.50E-06	1.26 [1.15, 1.39]	3.4 (P=0.65)	0 [0, 75]
rs7188458	16	88253985	G	A	G	1.00	9.48E-01	5.25E-03	0.42	0.32	0.46	0.07	1.44E-13	7.99E-11	1.63E-08	7.99E-11	1.34 [1.23, 1.46]	5.7 (P=0.33)	13 [0, 78]
rs459920	16	88258328	G	G	A	1.00	5.86E-01	2.57E-02	0.47	0.39	0.60	0.08	3.84E-19	5.41E-06	2.12E-04	5.41E-06	0.82 [0.75, 0.89]	7.5 (P=0.19)	33 [0, 73]
rs12918773	16	88268904	Imp	A	G	NA	-	-	-	-	-	-	-	1.34E-16	3.33E-16	1.34E-16	1.87 [1.62, 2.16]	-	-
rs258322	16	88283404	G	A	G	1.00	6.47E-02	1.80E-03	0.09	0.04	0.14	0.04	3.03E-14	7.54E-17	2.80E-16	7.54E-17	1.81 [1.58, 2.08]	3.4 (P=0.64)	0 [0, 75]
rs1800359	16	88332762	G	A	G	1.00	2.66E-01	6.19E-02	0.42	0.34	0.49	0.06	8.70E-12	1.41E-06	3.18E-06	1.41E-06	0.80 [0.74, 0.88]	7.8 (P=0.17)	36 [0, 74]
rs11861084	16	88403211	G	A	C	0.99	8.70E-01	2.52E-01	0.43	0.33	0.50	0.06	1.39E-11	1.64E-06	2.72E-06	1.64E-06	0.81 [0.74, 0.88]	8.2 (P=0.15)	39 [0, 76]
rs4408545	16	88571529	G	A	G	1.00	7.36E-02	1.15E-01	0.51	0.41	0.53	0.05	3.58E-06	1.29E-06	5.36E-06	1.29E-06	0.81 [0.74, 0.88]	3.9 (P=0.56)	0 [0, 75]
rs4238833	16	88578190	G	C	A	0.99	3.68E-01	1.02E-01	0.36	0.31	0.40	0.04	6.32E-07	1.04E-09	2.53E-09	1.04E-09	1.32 [1.21, 1.44]	2.5 (P=0.77)	0 [0, 75]
rs4785763	16	88594437	G	A	C	1.00	3.99E-01	6.75E-02	0.32	0.23	0.38	0.06	2.59E-09	1.68E-14	1.68E-14	2.84E-14	1.42 [1.30, 1.56]	9.0 (P=0.11)	44 [0, 78]
rs8059973	16	88607035	G	A	G	1.00	7.28E-01	8.18E-02	0.17	0.16	0.22	0.02	1.01E-01	6.81E-07	7.97E-06	6.81E-07	0.74 [0.65, 0.83]	1.1 (P=0.96)	0 [0, 75]

Supplementary Table 3. Detailed replication results. The results of the Cochran-Armitage trend test analysis of the replication samples for SNPs in regions identified in the genome-wide analysis and chosen for follow-up (Supplementary Table 2, chromosome 2 was not followed up). Results are shown for stratified analysis (by geographical location) for the replication samples. The table shows the results by replication sample set (GenoMEL genotyped at CNG or Leeds (genotyped in Leeds)), replication total (replication sets combined), and overall in combination with the genome-wide analysis. The OR is the per-allele odds ratio. Results marked with a dash sign were either not attempted or the assay did not work in the genotyping laboratory. HWE is the Hardy-Weinberg Equilibrium p-value calculated for controls and MAF is the minor allele frequency in controls. We also list results for SNPs on chromosome 20 and 22 that have been previously reported to be associated with melanoma (chromosome 20 in proximity to ASIP and chromosome 22 for nevi by Falchi et al [1]). Note that some SNPs were not genotyped in the genome-wide sample, so results for the last two datasets is in some cases the same. rs17305573 and rs4911442 were not genotyped in the GenoMEL replication set. Analyses including and excluding the one non-European population were conducted without qualitatively changing the results (see Supplementary Information). Note that for some SNPs, the minor allele differs from that presented in Table 1 and Supplementary Table 2; this reflects that the opposite strand has been assayed between the genome-wide array and the replication (Taqman) technology.

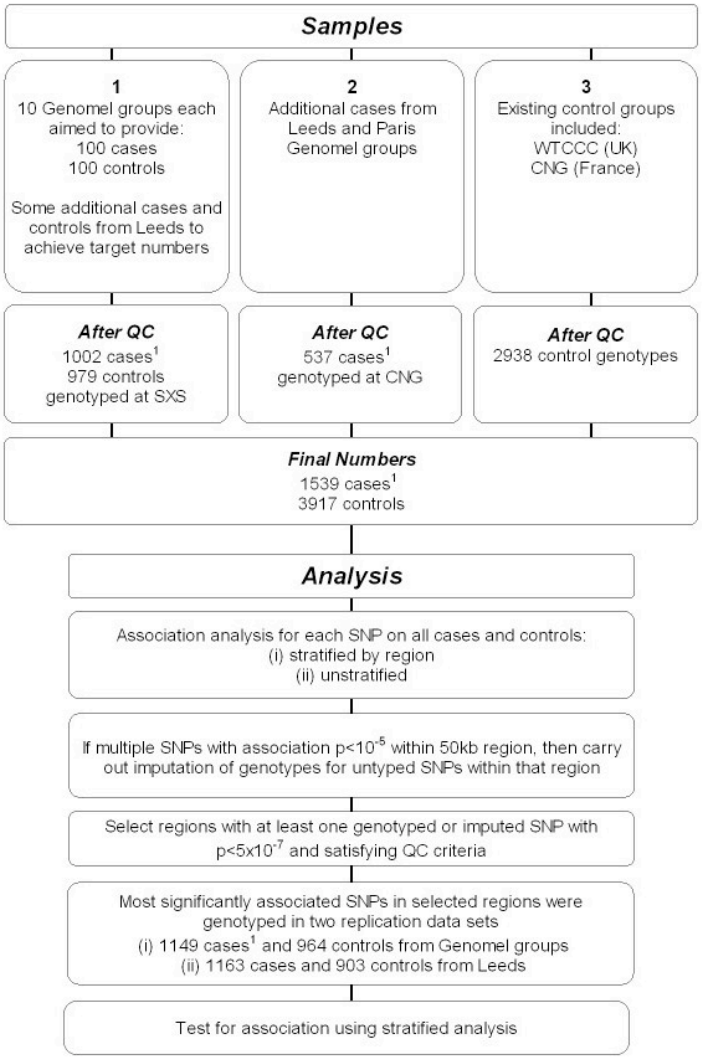
SNP	Chromosome	Position (bp)	Genomel Replication set		Leeds case-control		REPLICATION 1: Genomel Replication set		REPLICATION 2: Leeds case-control set		REPLICATION TOTAL: Genomel + Leeds case-control		OVERALL: Genome-wide + Replication	
			HWE	MAF	HWE	MAF	P-value	OR [Conf. Int.]	P-value	OR [Conf. Int.]	P-value	OR [Conf. Int.]	P-value	OR [Conf. Int.]
Chromosomal Regions Followed up on Basis of This Study														
rs6894498	5	95165802	0.49	0.11	3.10E-03	0.12	0.15	1.16 [0.95, 1.41]	0.93	0.99 [0.82, 1.20]	0.35	1.07 [0.93, 1.23]	0.35	1.07 [0.93, 1.23]
rs871775	5	95184524	0.50	0.12	1.60E-04	0.14	0.41	1.08 [0.90, 1.31]	0.27	0.90 [0.75, 1.08]	0.82	0.98 [0.86, 1.12]	1.01E-03	1.16 [1.06, 1.27]
rs4636294	9	21737803	0.64	0.49	0.14	0.51	0.037	0.87 [0.76, 0.99]	0.33	0.94 [0.82, 1.07]	0.032	0.90 [0.83, 0.99]	1.97E-06	0.86 [0.81, 0.92]
rs2218220	9	21746089	0.71	0.49	0.12	0.51	0.089	0.89 [0.79, 1.02]	0.25	0.93 [0.81, 1.06]	0.046	0.91 [0.83, 1.00]	6.40E-06	0.87 [0.82, 0.92]
rs7023329	9	21806528	0.92	0.48	0.07	0.50	0.096	0.89 [0.78, 1.02]	0.13	0.90 [0.79, 1.03]	0.023	0.90 [0.82, 0.99]	4.03E-07	0.85 [0.80, 0.91]
rs1042602	11	88551344	0.96	0.38	0.50	0.36	0.097	0.89 [0.78, 1.02]	0.27	0.93 [0.82, 1.06]	0.054	0.91 [0.83, 1.00]	0.011	0.92 [0.87, 0.98]
rs1393350	11	88650694	0.10	0.24	0.76	0.28	4.90E-06	1.40 [1.21, 1.62]	3.23E-03	1.22 [1.07, 1.40]	1.38E-07	1.30 [1.18, 1.44]	2.41E-14	1.29 [1.21, 1.38]
rs1126809	11	88657609	0.19	0.26	0.65	0.29	7.16E-05	1.34 [1.16, 1.54]	3.19E-03	1.22 [1.07, 1.40]	1.17E-06	1.27 [1.16, 1.40]	1.17E-06	1.27 [1.16, 1.40]
rs1847142	11	88661222	-	-	0.37	0.32	-	-	6.48E-04	1.26 [1.10, 1.44]	6.48E-04	1.26 [1.10, 1.44]	6.48E-04	1.26 [1.10, 1.44]
rs10830253	11	88667691	0.03	0.29	0.60	0.32	1.57E-03	1.26 [1.09, 1.45]	6.26E-04	1.26 [1.10, 1.43]	2.81E-06	1.26 [1.14, 1.39]	2.81E-06	1.26 [1.14, 1.39]
rs7188458	16	88253985	0.25	0.40	-	-	1.20E-03	1.25 [1.09, 1.42]	-	-	1.20E-03	1.25 [1.09, 1.42]	1.16E-12	1.30 [1.21, 1.40]
rs258322	16	88283404	0.01	0.09	0.58	0.12	8.20E-05	1.49 [1.22, 1.83]	3.38E-07	1.59 [1.33, 1.90]	1.07E-10	1.55 [1.36, 1.77]	2.54E-27	1.67 [1.52, 1.83]
rs4785763	16	88594437	0.87	0.33	0.83	0.36	4.05E-05	1.33 [1.16, 1.52]	2.53E-04	1.28 [1.12, 1.45]	5.13E-08	1.30 [1.18, 1.43]	5.96E-22	1.36 [1.28, 1.45]
Chromosomal Regions Considered for Followup on Basis of Other Studies														
rs910873	20	32635433	0.65	0.07	0.82	0.09	6.44E-05	1.61 [1.27, 2.03]	6.76E-05	1.52 [1.24, 1.86]	1.92E-08	1.55 [1.33, 1.81]	1.92E-08	1.55 [1.33, 1.81]
rs17305573	20	32643813	-	-	0.84	0.09	-	-	3.95E-05	1.53 [1.25, 1.87]	3.95E-05	1.53 [1.25, 1.87]	3.95E-05	1.53 [1.25, 1.87]
rs4911442	20	32818707	-	-	0.95	0.12	-	-	1.80E-05	1.48 [1.24, 1.77]	1.80E-05	1.48 [1.24, 1.77]	1.80E-05	1.48 [1.24, 1.77]
rs1885120	20	33040650	0.24	0.07	0.38	0.08	3.36E-04	1.55 [1.22, 1.97]	9.28E-06	1.61 [1.30, 1.99]	1.25E-08	1.58 [1.35, 1.85]	1.25E-08	1.58 [1.35, 1.85]
rs2284063	22	36874244	0.07	0.35	0.89	0.38	0.2648	0.92 [0.81, 1.06]	5.44E-06	0.73 [0.64, 0.84]	6.45E-05	0.82 [0.75, 0.90]	2.40E-09	0.83 [0.78, 0.88]
rs6001027	22	36875565	0.03	0.34	0.39	0.36	0.1605	0.90 [0.78, 1.04]	0.0030	0.81 [0.71, 0.93]	0.0023	0.86 [0.78, 0.95]	1.94E-08	0.83 [0.78, 0.89]

Supplementary Table 4. The top half of the table shows the results of a multiple stepwise regression analysis. We applied stepwise logistic regression to determine from among the multiple SNPs on chromosomes 9, 11 and 16 associated with melanoma risk, those which produced the strongest independent associations. These analyses were conducted independently by locus and also combining all regions. The table shows the results for the combination of all genetic regions, which are qualitatively identical to the analyses of each genetic region separately. Analyses are based on multiple logistic regression adjusting for geographical region. These analyses are restricted to individuals with complete genotyping across all SNPs; inclusion of individuals with incomplete genotyping for each locus made no difference except that for chromosome 9, in some analyses rs4636294 replaced rs7023329 as being the selected SNP. The table also lists the gene/genes closest to the SNP. The step number is the order that the SNP entered the stepwise model.

The bottom half of the table shows the results of an investigation of locus x locus interaction. Results are shown for logistic regression analysis adjusting for geographical location and including the top SNP from each of the 3 replicated regions in this study. Analysis involves 4959 persons with complete genotype information. The table shows the estimated effect sizes under the logistic regression model, the 95% confidence interval of the estimate and the corresponding p-value. Model 1 includes pairwise interactions between each pair of SNPs; none of the interactions approach significance. Comparison with Model 2, which does not include any interactions (similar to the top half of the table), shows only a marginally poorer fit (chi-square test statistic from likelihood ratio test = 2.13 with 3 df, p = 0.55) indicating there is no evidence of deviation from independence of the association with the 3 loci.

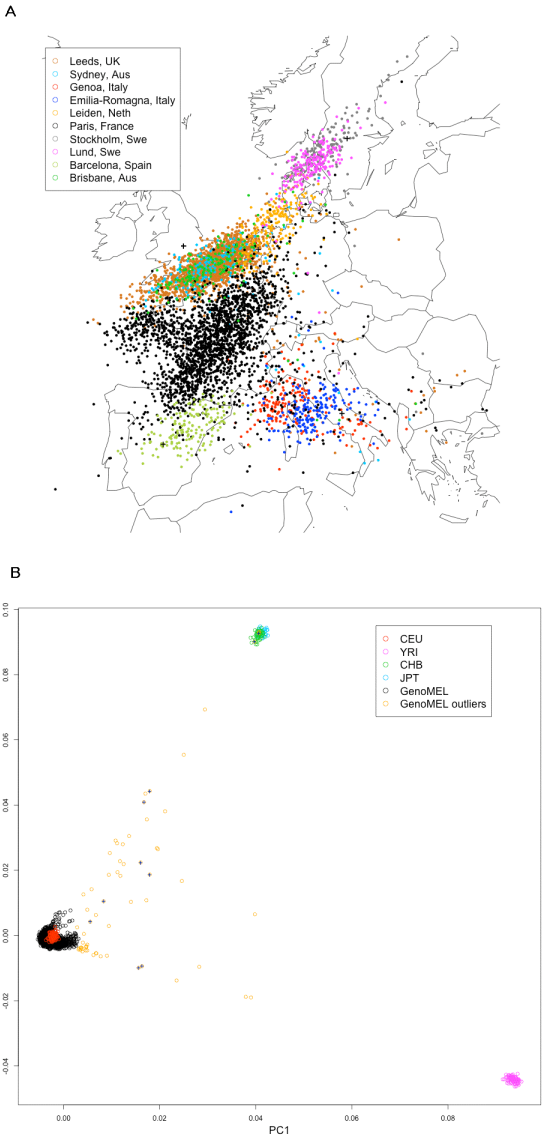
SNP(s)	**Chromosome(s)**	**Position (bp)**	**Gene**	**Multiple Regression: P to enter**	**Multiple regression: P in final model**
rs258322	16	88283404	CDK10 intron	8.3×10^{-15}	2.8×10^{-7}
rs1393350	11	88650694	TYR intron	2.6×10^{-7}	1.8×10^{-7}
rs4785763	16	88594437	Flanking 3'UTR of AFG3L1	5.3×10^{-7}	1.9×10^{-8}
rs8059973	16	88607035	Flanking 5'UTR of DBNDD1	5.3×10^{-7}	4.4×10^{-7}
rs7023329	9	21806528	MTAP intron	7.3×10^{-5}	9.3×10^{-5}
rs1011970	9	22052134	Flanking 5'UTR of CDKN2B	1.2×10^{-4}	1.2×10^{-4}
		Model 1: including all 2-way interactions		**Model 2: without any interaction**	
		OR [Conf. Int]	**P**	**OR [Conf. Int]**	**P**
rs7023329	9	0.83 [0.73, 0.95]	8.06×10^{-3}	0.82 [0.75, 0.90]	4.65×10^{-5}
rs1393350	11	1.24 [1.05, 1.48]	1.22×10^{-2}	1.30 [1.18, 1.43]	2.16×10^{-7}
rs258322	16	1.95 [1.50, 2.53]	5.77×10^{-7}	1.75 [1.52, 2.01]	3.72×10^{-15}
rs7023329 x rs1393350	9 and 11	1.04 [0.90, 1.19]	0.62	-	-
rs7023329 x rs258322	9 and 16	0.88 [0.72, 1.07]	0.19	-	-
rs1393350 x rs258322	11 and 16	1.03 [0.84, 1.28]	0.75	-	-
Model log-likelihood (df)		**-2718.7 (df=11)**		**-2719.8 (df=8)**	
Likelihood-Ratio test of model 1 versus model 2		**chi-squared (3 df) = 2.13 (p-value=0.55)**			

Supplementary Figure 1. An overview of study design including the two stage design involving a genome-wide phase followed by a replication phase in further independent samples. Samples were obtained from participating GenoMEL groups and included likely genetically -enriched cases and controls. Samples were genotyped at SXS (ServiceXS, Leiden, The Netherlands) and CNG (Centre National de Génotypage, Paris, France). Genotyping information on controls was obtained from the WTCCC (UK) and from CNG (Paris) to increase the power of the study. Following quality control (QC) which involved excluding samples on the basis of their likely non-European ancestry and samples with low call rates, statistical analysis involving both stratified and unstratified methods was conducted to identify regions putatively containing melanoma susceptibility genes. Follow-up in the replication phase included further GenoMEL cases and controls and population-based cases and controls from Leeds which had not been genotyped in the genome-wide phase.



[1]Cases preferentially selected for family history, multiple primaries or early age at onset as described in text

Supplementary Figure 2. A) Plot of fitted values after regressing latitude and longitude of centres on first two principal components (PC1 and PC2). Results shown for study data after QC, excluding those samples declared to be non-European. GenoMEL centres indicated by colour: Leeds (brown), Leiden (orange), Stockholm (grey), Lund (magenta), Paris (black), Barcelona (light green), Genoa (red), Emilia-Romagna (dark blue), Brisbane (dark green), Sydney & AMFS (reported as "Sydney" here, light blue). B) Principal Components for the genome-wide study and HapMap. Plot of first two principal components from analysis of study data (after QC) combined with HapMap data. Ethnicity of HapMap samples indicated by colour: Africa (YRI) in magenta, Japan (JPT) in blue, China (CHB) in green and Europe (CEU) in red. Study samples declared to be non-European (GenoMEL outliers) are coloured orange and those later confirmed to be of non-European ethnicity from records indicated by '+'. The remaining GenoMEL study samples assumed to be of European origin are coloured black.



Supplementary Figure 3. Chromosome 9p21. The map shows the relative locations of *MTAP* together with the three SNPs (rs7023329, rs2218220 and rs4636294) associated with melanoma risk in this analysis. These three SNPs are also associated with nevus number in an accompanying manuscript[1]. Further, multiple regression analysis showed independent contributions of SNPs on either side of CDKN2A, particularly for rs7023329 and rs1011970 (Supplementary Table 4). Other associations in the region include rs10757278 with coronary heart disease [4] and rs10811661 with type 2 diabetes [5]