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Genome-wide association study identifies three new melanoma susceptibility loci

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We report a genome-wide association study for melanoma that was conducted by the GenoMEL Consortium. Our discovery phase included 2,981 individuals with melanoma and 1,982 study-specific control individuals of European ancestry, as well as an additional 6,426 control subjects from French or British populations, all of whom were genotyped for 317,000 or 610,000 single-nucleotide polymorphisms (SNPs). Our analysis replicated previously known melanoma susceptibility loci. Seven new regions with at least one SNP with $P < 10^{-5}$ and further local imputed or genotyped support were selected for replication using two other genome-wide studies (from Australia and Texas, USA). Additional replication came from case-control series from the UK and The Netherlands. Variants at three of the seven loci replicated at $P < 10^{-3}$: an SNP in *ATM* (rs1801516, overall $P = 3.4 \times 10^{-9}$), an SNP in *MX2* (rs45430, $P = 2.9 \times 10^{-9}$) and an SNP adjacent to *CASP8* (rs13016963, $P = 8.6 \times 10^{-10}$). A fourth locus near *CCND1* remains of potential interest, showing suggestive but inconclusive evidence of replication (rs1485993, overall $P = 4.6 \times 10^{-7}$ under a fixed-effects model and $P = 1.2 \times 10^{-3}$ under a random-effects model). These newly associated variants showed no association with nevus or pigmentation phenotypes in a large British case-control series.

Cutaneous melanoma is predominantly a disease of fair-skinned individuals. Risk factors include family history[1], certain pigmentation phenotypes (notably the presence of fair skin, blue or green eyes, blond or red hair, sun sensitivity or an inability to tan[2–5]) and increased numbers of melanocytic nevi[6,7]. We previously reported phase 1 of a genome-wide association study (GWAS) of melanoma based on the Illumina 317k array[8]. Data from this study reinforced the importance of genetically determined melanoma-associated phenotypes by identifying the major common genetic determinants of risk in the populations considered: the *MC1R* locus (associated with red hair, freckling and sun sensitivity)[4,5,9,10], tyrosinase (*TYR*) gene variants that encode skin color[11] and a region near *CDKN2A* and *MTAP* that is associated with number of melanocytic nevi[8,12]. Furthermore, we confirmed the importance of a haplotype spanning the agouti signaling protein (*ASIP*) locus[11,13] and a second locus at 22q13 determining nevus count variation, which was identified by a GWAS of nevus count[12].

Both phase 1 and phase 2 of this study were carried out by the GenoMEL Consortium, a collaboration focusing on genetic susceptibility to melanoma. The study used samples collected by GenoMEL participants across populations of European ancestry living at different latitudes. In total, 14 GenoMEL groups contributed DNA samples from individuals with melanoma and control individuals of European (or Israeli) ancestry (**Supplementary Table 1**). Phase 1 was based on 1,650

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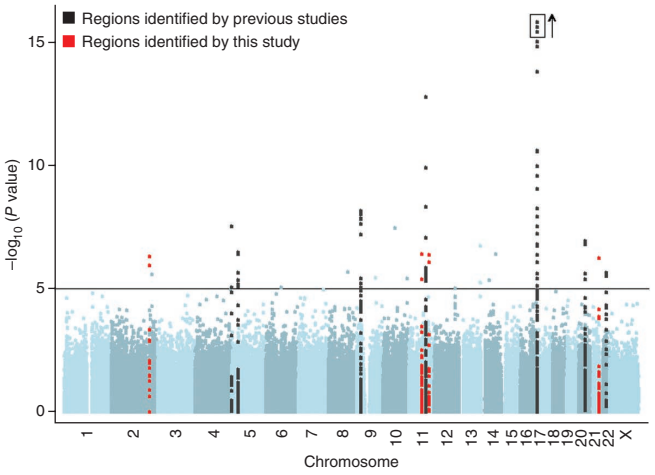

Figure 1 Manhattan plot of results of Cochran-Armitage (CA) trend test stratified by geographic region with $-\log_{10}$ *P* values shown. The solid horizontal line indicates a *P* value of 10^{-5}. Markers within 50 kb of an SNP associated with melanoma are marked in black for those identified in a previous GWAS and replicated here, and in red for those first identified in the current study. The *y* axis is truncated at $P = 10^{-15}$, although three SNPs in the *MC1R* region have stronger *P* values up to 2.7×10^{-27}, as signified by the box and arrow.

subjects with melanoma from Australian and European populations chosen because they had a phenotype argued to enrich for genetic susceptibility (early onset, multiple primary melanomas or modest family history of melanoma). In phase 2, a further 1,523 individuals with melanoma (1,211 of whom are genetically enriched: 532 with a family history, 277 with multiple primaries but no family history and 402 with early disease onset but no multiple primaries or family history) and 1,112 control individuals were genotyped using the denser Illumina 610k array (**Supplementary Note**). To optimize power, we combined the data from the two phases and performed an overall analysis. The Australian data used in phase 1 were dropped from the combined phase 1 and phase 2 analysis because these samples are included in the Australian GWAS that formed one of the replication studies. After quality control was applied to SNPs and samples (**Supplementary Note**), including principal-component analysis (PCA) to identify samples of non-European ancestry (**Supplementary Fig. 1**), the analysis used 2,804 subjects with melanoma (2,692 European and 112 Israeli individuals) and 1,835 control subjects from GenoMEL studies and 5,783 control subjects from France and the UK Wellcome Trust Case-Control Consortium (WTCCC). A trend test, stratified by geographical region, was applied to each SNP (**Fig. 1**; and see Online Methods). Little evidence was found of population stratification ($\lambda = 1.06$, **Supplementary Note**).

Strong evidence was found for previously identified loci (**Supplementary Figs. 2**, **3** and **Supplementary Table 2**)[8,11–18] and for another pigmentation gene, *SLC45A2*, already reported to be associated with melanoma risk[15]. The protein encoded by *SLC45A2* is involved in melanosome maturation and pigmentation. The rs35390 SNP identified here is associated with melanoma[15] and with variation in hair color[15,19], in accordance with the observed pattern of known melanoma pigmentation risk factors[2–5].

We also confirmed a role for the rs401681 SNP in the region of *TERT* and *CLPTM1L*, which has also previously been shown to modify melanoma risk[18] (**Supplementary Figs. 2**, **3** and **Supplementary Table 2**)[8,11–16,20]. The confirmation of this SNP association follows reported associations of variants at this locus with risk of basal cell carcinomas, hematological malignancies and cancer of the bladder, cervix, lung, pancreas and prostate[18,21]. It was originally reported that the pattern of risk for melanoma was in the opposite direction to that for other cancers[18], and we confirm this observation.

Seven further regions showed evidence of association with melanoma susceptibility (**Table 1** and **Supplementary Table 3**). We sought replication in two other GWAS for the SNPs with the strongest evidence, preferentially considering SNPs common to all arrays. In regions with no SNP common to all platforms, we selected for follow-up both our top genotyped SNPs and the most significant imputed SNPs that had been genotyped in the replication studies. Further, these SNPs were genotyped in a replication sample set from the UK and The Netherlands (1,579 individuals with melanoma and 2,036 control subjects in total, **Supplementary Note**). The evidence from both the hypothesis-generating and replication data sets are provided (**Table 1**). Of these seven regions, three (on chromosomes 2 (rs13016963), 11 (rs1801516) and 21 (rs45430)) showed strong evidence of replication ($P < 10^{-3}$), three (on chromosomes 2 (rs10932444), 12 (rs7139314) and 13 (rs9515125)) showed no evidence of replication, and one (on chromosome 11 (rs1485993)) showed marginal evidence of replication. Three of the loci showed overall combined evidence of association at $P < 5 \times 10^{-8}$, as indicated by fixed-effects meta-analysis.

The *CASP8* region (chromosome 2) contains a number of SNPs showing evidence of association with melanoma risk; because of a lack of overlap in the SNPs across arrays, we report multiple SNPs that were either genotyped or imputed across platforms, all of which show evidence of association (**Fig. 2**, **Table 1** and

Table 1 Summary of results from this study for the four regions showing evidence of replication

					GenoMEL genome-wide		Replication samples (genotyped + imputed)		Genome-wide plus replication samples (genotyped + imputed)		Candidate gene
SNP	Chromosome	Position	Allele	MAF	OR	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	
rs13016963	2	201852173	A	0.37	1.18	5.68×10^{-7}	1.11 (1.06, 1.18)	9.2×10^{-5}	1.14 (1.09, 1.19)	8.6×10^{-10}	*CASP8*
rs1485993	11	69071595	A	0.37	1.19	4.15×10^{-7}	1.07 (1.01, 1.13)	0.017	1.11 (1.04, 1.18)[a]	0.0012	*CCND1*
rs1801516	11	107680672	A	0.13	0.79	4.80×10^{-7}	0.87 (0.81, 0.94)	3.4×10^{-4}	0.84 (0.79, 0.89)	3.4×10^{-9}	*ATM*
rs45430	21	41667951	G	0.39	0.85	5.60×10^{-7}	0.91 (0.86, 0.96)	4.2×10^{-4}	0.88 (0.85, 0.92)	2.9×10^{-9}	*MX2*

Each SNP under consideration, their position (in bp) and minor allele frequency (MAF); the per-allele OR (based on the minor allele) and *P* value are given for this GWAS, for the meta-analysis of the replication data sets (from the Houston GWAS, the Australian GWAS, and the UK and The Netherlands replication samples) and for the combined genome-wide and replication analyses. The Houston GWAS and the Australian study both used a different array from the current study for at least some samples; therefore, some of their results presented here include imputed data. Further genotyping was conducted in the UK and The Netherlands replication samples for SNPs with positive support from the GWAS replication data. All meta-analyses are based on a fixed-effects model with the exception of those for *CCND1*, in which random-effects analysis was used because of the observed heterogeneity. See **Supplementary Table 3** for an extended version of this table.

[a]Random-effects analysis used.

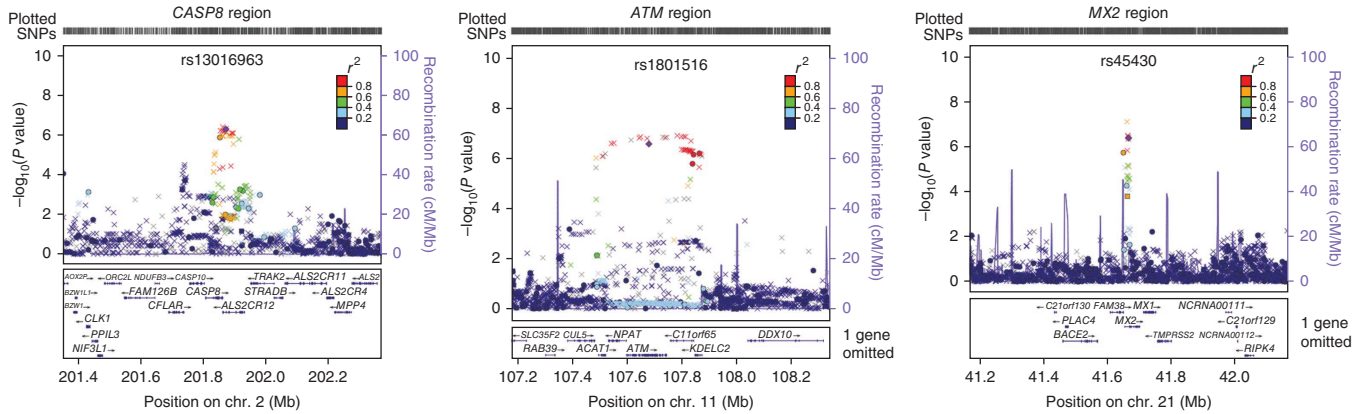

Figure 2 Stratified CA trend tests for the three replicated regions on chromosomes 2, 11 and 21. The $\log_{10}$ *P* values are from the CA trend test (stratified by geographical region) for genotyped and imputed SNPs, as indicated on the left-hand vertical axis. SNPs genotyped for all samples are plotted as circles, SNPs imputed for all samples as crosses and SNPs genotyped for some samples and imputed for others (as a result of chip differences) as squares. The most significant genotyped SNP is colored purple (with its name above), and the degree of LD between that SNP and the others is indicated by color according to the key (red being the greatest degree of LD). The estimated recombination rate is given by the blue line and indicated on the right-hand vertical axis. The genes in the region and their positions are given underneath the graph. Plots were produced using LocusZoom[27].

Supplementary Table 3). The strongest evidence of association for a single SNP is from rs700635 ($P = 2.4 \times 10^{-9}$, odds ratio (OR) = 1.15 overall). All the SNPs are in the region of the *CASP8* gene, which encodes a member of a family of proteases. These proteins have a critical role in the control of cell proliferation and induce apoptotic cell death, which make them candidate cancer susceptibility genes. A recent meta-analysis[22] of three polymorphisms in *CASP8* found that individuals with one or more copies of the variant encoding a D302H substitution have a decreased risk of multiple types of cancer. In this study, the variant encoding D302H could be imputed, but it showed only marginal evidence of association ($P = 0.05$), suggesting that this is not a variant associated with melanoma. The evidence for melanoma risk was consistent across populations (**Fig. 3**).

The rs1801516 SNP in *ATM* (chromosome 11) (**Fig. 2**, **Table 1** and **Supplementary Table 3**) is a missense mutation (c.5557 > A, encoding a D1853N substitution) in a gene that encodes a protein that repairs double-strand DNA breaks. An association has been postulated between *ATM* and a number of cancer types[23]. For melanoma, the A allele is protective ($P = 3.4 \times 10^{-9}$, OR = 0.84 overall). Overall, the evidence for melanoma is consistent across populations and case type, with no evidence of heterogeneity (**Fig. 3**).

The third replicated region around *MX2* (chromosome 21) showed consistent effect sizes across the replication data sets (**Fig. 2**, **Table 1** and **Supplementary Table 3**) and across populations (**Fig. 3**). The SNP that was pursued in the replication study is rs45430 ($P = 2.9 \times 10^{-9}$, OR = 0.88 overall), which is intronic to *MX2* and has not previously been associated with cancer susceptibility.

A fourth region, adjacent to *CCND1* (chromosome 11), a proto-oncogene that is a key regulator of cell cycle progression, showed consistent effect sizes across all the replication sets (**Table 1**, **Supplementary Fig. 4** and **Supplementary Table 3**), with best overall replication *P* values of 0.011 for rs11263498 and 0.017 for rs1485993. However, the replication sets produced a notably smaller OR (for example, for rs11263498, 1.08) than the discovery set (1.19) (proportion of variation due to heterogeneity (I^2) = 0.507). This finding is potentially the result of the well known 'winner's curse' effect[24] that causes the initial discovery set to overestimate the OR, leading in turn to a discrepancy between the overall *P* value based on fixed-effects and random-effects meta-analysis ($P = 1.7 \times 10^{-7}$ and $P = 0.00046$, respectively, for rs11263498). Thus, although we have strong support for an involvement of this region in melanoma, the evidence cannot be considered conclusive (see **Supplementary Note** for further details). However, further support comes from the interim analysis of a recently completed melanoma GWAS of 494 individuals with melanoma and 5,628 control individuals from the Nurses' Health Study and Health Professionals Follow-up Study

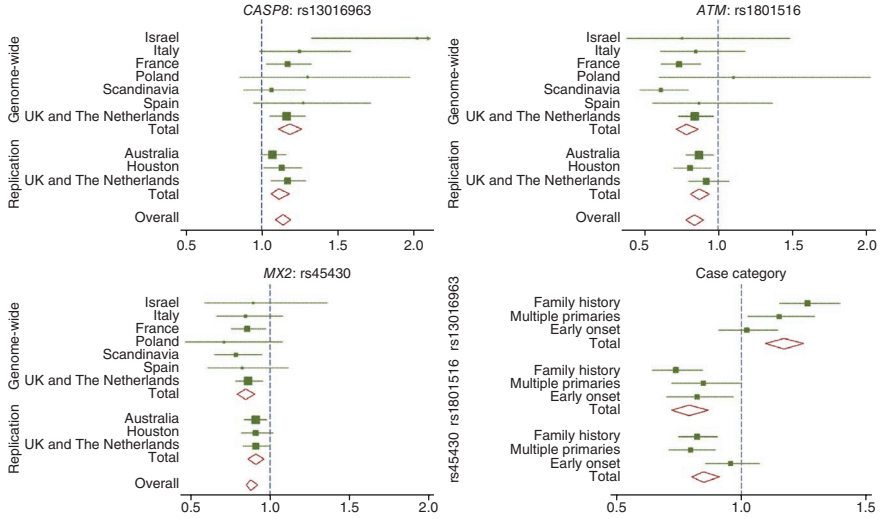

Figure 3 Forest plot of the per-allele OR for melanoma for SNPs in the three regions first identified by this study. Plots show the current evidence for effects by geography and by case type (family history, multiple primaries or early onset) in the genome-wide and replication samples.

Table 2 Summary of results for nevus count, pigmentation and melanoma analyses from the Leeds case-control samples examining the 11 SNPs replicated for melanoma association in this or previous studies

Chromosomal region	Candidate gene	SNP	MAF	Percentage of variation in log nevus count explained by SNP		Percentage of variation in pigmentation explained by SNP		Per-allele OR (95% CI) for risk of melanoma	OR (95% CI) for risk of melanoma with one copy of minor allele	OR (95% CI) for risk of melanoma with two copies of minor allele
				r^2	P	r^2	P			
2q33-q34	*CASP8*	rs13016963	0.33	0.21	0.083[a]	0.05	0.33	1.25 (1.07, 1.46)	1.26 (1.01, 1.56)	1.56 (1.11, 2.18)
5p15.33	*TERT-CLPTM1L*	rs401681	0.46	0.50	**0.0070**	0.13	0.11	1.08 (0.93, 1.25)	1.15 (0.90, 1.47)	1.15 (0.85, 1.55)
5p13.2	*SLC45A2*	rs16891982	0.03	0.02	0.62	1.33	**1.9×10^{-6}**	0.72 (0.44, 1.18)	0.78 (0.47, 1.30)	NA
6p25-p23	*IRF4*	rs12203592	0.24	0.21	0.084	2.76	**5.6×10^{-12}**	0.80 (0.67, 0.95)	0.72 (0.58, 0.91)	0.81 (0.49, 1.35)
9p21	*CDKN2A/MTAP*	rs7023329	0.49	0.29	**0.047**	0.02	0.55	0.86 (0.73, 1.00)	0.62 (0.47, 0.82)	0.73 (0.53, 1.01)
11q14-q21	*TYR*	rs1393350	0.27	0.00	0.95	1.07	**2.0×10^{-5}**	1.34 (1.14, 1.58)	1.19 (0.96, 1.49)	2.12 (1.41, 3.19)
11q22-q23	*ATM*	rs1801516	0.14	0.07	0.33	0.00	0.95	0.88 (0.71, 1.09)	0.93 (0.73, 1.19)	0.59 (0.29, 1.21)
16q24.3	*MC1R*	rs258322	0.10	0.00	0.81	4.00	**9.0×10^{-17}**	1.83 (1.44, 2.32)	1.71 (1.33, 2.22)	7.14 (1.70, 29.98)
20q11.2-q12	*ASIP*	rs4911442	0.13	0.07	0.34	0.93	**8.2×10^{-5}**	1.35 (1.08, 1.68)	1.32 (1.03, 1.69)	2.06 (0.85, 5.00)
21q22.3	*MX2*	rs45430	0.38	0.00	0.80	0.05	0.32	0.90 (0.77, 1.05)	0.97 (0.77, 1.22)	0.77 (0.56, 1.07)
22q13.1	*PLA2G6*	rs6001027	0.37	0.39	**0.018**	0.12	0.16	0.78 (0.66, 0.91)	0.79 (0.63, 0.90)	0.60 (0.42, 0.84)
	Total			2.33		9.83				

Results are shown for the proportion and significance of log nevus count variation explained by each SNP, adjusted for age and sex among subjects with melanoma and control subjects (adjusted for case-control status), the proportion and significance of case-control adjusted pigmentation variation score explained by each SNP, where the score is calculated from factor analysis of six correlated pigmentation phenotypes (Online Methods), and the association with melanoma risk (both as per-allele OR with 95% CI and by genotype (compared to a baseline of the homozygote for the common allele)). Bold type indicates P values <0.05.

[a] $P = 0.004$ for controls only.

(OR = 1.18 for rs1485993, $P = 0.014$, unpublished data). This locus therefore remains a strong candidate, being well known in melanoma carcinogenesis[25].

In phase 1 of the study, all melanoma susceptibility loci identified were associated with either skin pigmentation or nevus count variation[8]. For study subjects from Leeds, UK, detailed nevus count and pigmentation information has been obtained for individuals with melanoma and control individuals[26]. The association between nevus count, pigmentation and all SNPs associated with melanoma are shown (**Table 2**). (Note that not all SNPs show convincing evidence of melanoma association within the Leeds case-control samples, reflecting limited power.) As expected, *MC1R*, *SLC45A2*, *IRF4* and *TYR* are confirmed to be associated with pigmentation, and the rs4911442 SNP on chromosome 20 shows strong association with pigmentation, providing further support for *ASIP* as a candidate gene at this locus and implicating probable linkage disequilibrium (LD) with variants within an *ASIP* regulatory region. SNPs in the region of *CDKN2A/MTAP* and *PLA2G6* are associated with nevus variation. The *CLPTM1L* SNP is found in the region of *TERT* and *CLPTM1L* and is also associated with nevus count variation, suggesting its effect on melanoma risk modification may be via this mechanism. We previously showed that *IRF4* had a complex relationship with nevus count and melanoma risk[14], and there are suggestions for SNPs in the *CASP8* region of a relationship between genotype and nevus count in control individuals; among individuals with melanoma, the association is not apparent (**Table 2**). Finally, the SNPs in the *ATM* and *MX2* regions show no association with either nevus count or pigmentation, suggesting alternative, unknown mechanisms, although these variants require evaluation in other populations (**Supplementary Note**).

Overall, we report three loci newly associated with melanoma risk, which achieve an overall significance level of 5×10^{-8} based on fixed-effects meta-analysis, and a potential fourth locus. The power to detect SNPs with effect sizes similar to those estimated from the replication studies is low, and we see many more SNPs in new regions (from across the genome) reaching P values between 10^{-4} and 10^{-5} than expected (68 with minor allele frequency (MAF) > 0.05 compared with an expected 46), suggesting that there may be many other genetic regions with a similar effect on melanoma risk (**Supplementary Note**). Currently, 11 loci have been identified (**Table 2**), with the suggestion that 5 of these regions act through the pigmentation phenotype and at least 3 through the nevus phenotype, reflecting the major phenotype-associated risk factors for melanoma. Of note, at least two of the newly identified loci appear to influence risk through a new mechanism, opening up potential new directions for melanoma research.

URLs. GenoMEL, http://www.genomel.org/; Epidemiological study on the Genetics and Environment of Asthma (EGEA), http://cesp.vjf.inserm.fr/egeanet/; Wellcome Trust Case Control Consortium, http://www.wtccc.org.uk/; QUANTO 1.1, http://hydra.usc.edu/gxe.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

J.H.B. and M.M.I. led and carried out the statistical analysis, contributed to the design of the study and were members of the writing team. M. Harland contributed to the design of the study and provided genotyping information. J.C.T. carried out statistical analyses and was a member of the writing team. J.F.A., P.A.A., L.A.A., B.K.A., M.-F.A., E.A., W.B., D.C., A.E.C., D.D., A.M.D., D.F.E., E.F., P. Ghiorzo, G.G.G., M. Hocevar, V.H., C.I., M.A.J., G.J., G.L., M.T.L., J. Lang, R.M., J.M., N.G.M., A.M., G.W.M., S.N., L.P., J.A.P.-B., R.T., N.v.d.S., J. Hansson and D.C.W. contributed to the identification of suitable samples for the study. B.B. contributed to the design of the study and supervised the initial processing of samples. G.B.-S., K.M.B., B.B.-deP., L.A.C.-A., T.D., D.E.E., J. Hansson, J.L.H., R.F.K., J. Lubiński, F.A.v.N., H.O., S.P. and P.V.B. contributed to the design of the study. H.S. and B.J. carried out genotyping and contributed to the interpretation of genotyping data. P. Galan, J.R.-M. and D.Z. contributed to the interpretation of genotyping data. J. Han contributed results of a confirmatory study. C.I.A., S.F., J.E.L. and Q.W. led and contributed analyses from the Houston study. N.K.H., G.J.M. and S.M. led and contributed results from the Australian study. G.M.L. provided genotyping information and contributed to the interpretation of genotype data. F.D., P.A.K., E.C., A.M.G. and E.M.G. advised on statistical analysis and contributed to the design of the study. N.A.G. was consortium deputy lead and contributed to the design of the study. J.A.N.B. was overall consortium lead and contributed to the design of the study. D.T.B. led the analysis group, contributed to the design of the study and was a member of the writing team.

COMPETING FINANCIAL INTERESTS

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ONLINE METHODS

Statistical analysis. *Subject recruitment.* Approval for these studies was obtained for each recruiting center. Informed consent was obtained from all participants.

PCA and population stratification. To identify individuals of non-European ancestry, SNPs were thinned to reduce LD and combined with the HapMap data on 1,207 individuals of wide-ranging ancestry. PCA was applied to these data using EIGENSTRAT[28,29], the first two principal components (PCs) clearly separating the HapMap data into distinct clusters according to ancestry (**Supplementary Fig. 1a**).

The remaining samples from individuals of European ancestry were analyzed similarly. Despite the fact that PCA makes no use of the geographical origin of the samples, plotting the first two PCs clearly grouped the samples by the center from which they were collected, with little overlap between centers (**Supplementary Fig. 1b**). This demonstrated that the majority of individuals were ancestrally from the regions where they were collected, and a few were clearly outliers from elsewhere in Europe. The third and fourth PCs seemed to separate out the samples from Israel and Poland (**Supplementary Fig. 1c**). Although the analysis with the HapMap data had showed these individuals to be of European descent, PCA indicated that these two groups were to some extent outliers from the majority of the GenoMEL samples.

Association analysis. The primary analysis was a Cochran-Armitage (CA) trend test, stratified by region (as defined in the **Supplementary Note**). Further analyses were applied to the most significantly associated SNPs to assist in interpretation of results: a stratified CA trend test excluding samples from Polish and Israeli individuals, a stratified CA trend test stratifying by region and study phase, and a logistic regression analysis adjusted for region and the first four PCs. The effect of these further adjustments on the results was modest (**Supplementary Note**) and may be attributable to a reduction in sample size, suggesting no problem with stratification. Equivalent 1-degree-of-freedom stratified trend tests were carried out for the X chromosome; males were treated as equivalent to homozygous females, and a variance estimate was used that allows for the different variance of male and female contributions[30].

Subgroup analyses of samples from the different subtypes of melanoma relative to all control subjects were also conducted for the most strongly associated SNPs.

Replication analysis. Regions were chosen for replication if a SNP had a $P < 10^{-5}$ from the primary association analysis, with at least one other SNP within 500 kb having $P < 10^{-4}$. All such regions were also imputed. (See **Supplementary Note** for details of imputation and replication analysis.)

The results of the stratified analysis are reported (**Table 1** and **Supplementary Table 3**). Replication *P* values are listed separately for each of the three replication panels. None of the regions showed significant heterogeneity between studies and none had I^2 large enough to cause concern: it has been suggested that values below 31% are of "little concern" and those above 56% should induce "considerable caution"[31]. However, the *CCND1* locus showed moderate heterogeneity (**Supplementary Table 3**). Thus, for the sake of caution, we applied a random-effects model to the meta-analysis of *CCND1* for all data combined and a fixed-effects model to the remaining meta-analyses. Here, the method of DerSimonian and Laird[32] was applied to estimate the between studies variance, $\hat{\tau}^2$. An overall random-effects estimate was then calculated using the weights $1/(v_i + \hat{\tau}^2)$, where v_i is the variance of the estimated effect. $\hat{\tau}^2 = 0.0$ for the fixed-effects analyses.

Analysis of nevus and pigmentation phenotype. For one SNP in each of the replicated regions, we examined the relationship between that SNP and nevus count and pigmentation (**Table 2**). Nevus and pigmentation phenotype data were available for 980 individuals with melanoma and 499 control individuals from the Leeds case-control study[26,33]; pigmentation data were available for additional individuals from the Leeds melanoma cohort study[34], giving at total of 1,458 subjects with melanoma and 499 control subjects. For each SNP, logged age- and sex-adjusted total nevus count was regressed on the number of risk alleles, adjusting for case-control status. A sun-sensitivity score was calculated for all subjects based on a factor analysis of six pigmentation variables (hair color, eye color, self-reported freckling as a child, propensity to burn, ability to tan and skin color on the inside upper arm)[32]. This score was similarly regressed on number of risk alleles and adjusted for case-control status. Power calculations for this analysis were carried out using Quanto[35]. Based on the total available sample of individuals with melanoma and controls, the study had 49% power to detect the effect of a gene explaining 0.25% of the variation in nevus count at a 5% significance level; there was 78% power to detect an effect explaining 0.5% of the variation and 97% power to detect an effect explaining 1% of the variation. Note that these percentages of variation correspond to a steeper slope in nevus count for a rarer SNP, such as rs1801516 in *ATM*, than for a more common SNP, such as rs401681 in the *TERT-CLPTM1L* locus. For the pigmentation analysis, in which a larger number of individuals were included, the corresponding powers are 60%, 88% and 99%, respectively. It is thus not possible to rule out a small effect on these phenotypes of the newly discovered melanoma SNPs, but a substantial effect (similar to the effect of the SNPs in *CLPTM1L* on pigmentation) is very unlikely to have been missed.

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