Updated Field Synopsis and Systematic Meta-Analyses of Genetic Association Studies in Cutaneous Melanoma: The MelGene Database

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We updated a field synopsis of genetic associations of cutaneous melanoma (CM) by systematically retrieving and combining data from all studies in the field published as of August 31, 2013. Data were available from 197 studies, which included 83,343 CM cases and 187,809 controls and reported on 1,126 polymorphisms in 289 different genes. Random-effects meta-analyses of 81 eligible polymorphisms evaluated in >4 data sets confirmed 20 single-nucleotide polymorphisms across 10 loci (TYR, AFG3L1P, CDK10, MYH7B, SLC45A2, MTAP, ATM, CLPTM1L, FTO, and CASP8) that have previously been published with genome-wide significant evidence for association (P < 5 × 10−8) with CM risk, with certain variants possibly functioning as proxies of already tagged genes. Four other loci (MITF, CCND1, MX2, and PLA2G6) were also significantly associated with 5 × 10−8 < P < 1 × 10−3. In supplementary meta-analyses derived from genome-wide association studies, one additional locus located 11 kb upstream of ARNT (chromosome 1q21) showed genome-wide statistical significance with CM. Our approach serves as a useful model in analyzing and integrating the reported germline alterations involved in CM.

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INTRODUCTION

The incidence of cutaneous melanoma (CM), currently the leading cause of skin cancer–related mortality, has increased substantially over the past several decades in populations of European descent (Nikolaou and Stratigos, 2014). About 5–10% of CM patients have a positive family history (Florell et al., 2005), and family studies in twins have estimated that ~55% of the phenotypic variance is attributable to inherited factors (Shekar et al., 2009). Although highly penetrant, causative mutations in single genes, e.g., in CDKN2A/CDK4 and BAP1, have been identified in mainly familial cases of cutaneous and cutaneous/uveal melanoma, respectively, they only account for a small proportion of all cases (Tsao et al., 2012; Hill et al., 2013). The majority of CM cases are likely caused by the interaction of a few environmental factors, with excessive exposure to UVR being the most prominent risk factor, and dozens to hundreds common genetic variants that individually exert moderate risk effects (Law et al., 2012; Meng et al., 2012).

Numerous genetic association studies have been published to date reporting on multiple polymorphisms as potential risk factors for CM, including seven genome-wide association studies (GWAS) on CM (Brown et al., 2008; Bishop et al., 2009; Amos et al., 2011; Barrett et al., 2011; Macgregor et al., 2011; Teerlink et al., 2012; Iles et al., 2013). This increasing amount of data is more and more difficult to follow-up and interpret. We previously created the freely available online MelGene database (www.melgene.org, Chatzinasiou et al., 2011), which provides a comprehensive qualitative overview of all published genetic association studies in CM and presents a meta-analysis of eligible polymorphisms that have been assessed in at least four independent case-control data sets.

In this study, we present a systematic field synopsis of the currently available epidemiological data in the field of...
genetic associations of CM on the basis of a comprehensive update of the MelGene database. To this end, we added all eligible genetic association studies that have been published within a time period of 37 months since our original publication (Chatzinasiou et al., 2011) and present an updated meta-analysis of polymorphisms investigated in CM. All eligible publications are cited at the online MelGene database.

RESULTS
Our PubMed literature search of 1,285 citations yielded a total of 197 eligible studies (17 GWAS and 180 candidate-gene studies), reporting on 1,126 single-nucleotide polymorphisms (SNPs) across 289 genes. This included 51 additional studies (43 candidate-gene studies and 8 GWAS) since our original publication (Chatzinasiou et al., 2011) and 16 loci that were included for the first time in our database. The median number of polymorphisms assessed per study was 4.5 (interquartile range: 2–16). The median sample size analyzed per polymorphism was 1,060 combined cases and controls (interquartile range: 411–10,168). The most frequently analyzed genes were MCIR (studied in 36 publications), ASIP (13 publications), TYR (13 publications), and VDR (14 publications). Eighty-one SNPs met the inclusion criteria for meta-analysis; 35 were derived from candidate-gene association studies only, whereas 46 had available data from both candidate-gene studies and GWAS and/or replication efforts of GWAS signals.

Twenty SNPs across 10 independent loci (i.e., \( r^2 < 0.30 \)) showed genome-wide significant evidence for association with CM risk (\( P < 5 \times 10^{-8} \)). Specifically, the most significantly associated SNPs per locus were rs10931936 in CASP8 (on chromosome 2q33.1), rs16891982 in SLC45A2 (5p13.3), rs401681 in CLPTM1L (5p15.33), rs7023329 in MTAP (9p21.3), rs1126809 in TYR (11q14.3), rs11201516 in ATM (11q22.3), rs4785763 in AFG3L1P (16q24.3), rs258322 in CDK10 (16q24.3), rs16953002 in AFG3L1P (16q24.3), rs16953002 in FTO (16q12.2), and rs1885120 in MYH7B (20q11.22; Table 1, Supplementary Table S1 online). This list includes three genome-wide significant loci, i.e., ATM, FTO, and CASP8, identified by recent studies (Barrett et al., 2011; Iles et al., 2013; Pena-Chilet et al., 2013) published since our last field synopsis (Chatzinasiou et al., 2011). Of note, rs4785763 and rs258322 were not in notable linkage disequilibrium with each other (\( r^2 = 0.20 \)); thus, they are counted as independent loci in our synopsis. The median summary odds ratio (OR) across all top independent genome-wide significant signals was 1.20 (95% confidence interval: 1.12–2.38). The median number of data sets for SNPs included in each meta-analysis was six and the median total sample size was 7,486.

In addition, meta-analyses across 24 SNPs yielded nominally significant evidence for association with CM risk (\( 5 \times 10^{-8} < P < 0.05 \)). Four loci showed strong but sub-genome-wide significant evidence for association (i.e., \( 5 \times 10^{-8} < P < 1 \times 10^{-3} \)): rs149617956 in MITF (3p13, OR = 2.34, \( P = 2.5 \times 10^{-4} \)), rs11263498 in CCND1 (11q13.3, OR = 1.11, \( P = 4.6 \times 10^{-4} \)), rs6001027 in PLA2G6 (22q13.1, OR = 0.86, \( P = 7.54 \times 10^{-3} \)), and rs45430 in MX2 (21q22.3; OR = 0.89, \( P = 1.07 \times 10^{-5} \)). Both rs11263498 and rs45430 showed “weak” credibility (grade “C”), whereas the association result of rs6001027 and rs149617956 showed only “moderate” credibility (grade “B”). The latter result is due to the fact that this variant has a low minor allele frequency (i.e., MAF = 0.6%; Supplementary Table S1 online); thus, the “amount of evidence” for this meta-analysis is limited, whereas the meta-analysis shows consistent effect size estimates (\( P = 0, 95\% \) confidence interval: 0–85) across all four included data sets and no indication of potential bias (Ioannidis et al., 2007). The grading of the 20 remaining SNPs yielding nominally significant but statistically weaker evidence for association with CM risk upon meta-analysis showed “strong” (grade “A”) epidemiologic credibility for 3, “moderate” (grade “B”) epidemiologic credibility for 4, and “weak” (grade “C”) epidemiologic credibility for 13 variants (Supplementary Table S1 online).

Supplementary meta-analysis
In addition to the main analysis on variants for which data of at least four independent data sets were available, we performed supplementary meta-analysis on variants for which only three data sets derived from GWAS and/or GWAS replication data sets were available (Supplementary Table S2 online). Upon meta-analysis of 72 SNPs, a polymorphism located ~11 kb upstream of ARNT on chromosome 1q21 (rs7412746; Macgregor et al., 2011) showed genome-wide significant evidence for association with CM risk (OR = 0.87, \( P = 9 \times 10^{-11} \); Supplementary Table S2 online).

Additional variants with genome-wide significance reported in <3 data sets (not subjected to meta-analysis)
Furthermore, CM GWAS reported on additional SNPs across two independent loci that showed genome-wide significant evidence for association (Bishop et al., 2009; Amos et al., 2011; Teerlink et al., 2012); however, these results were based on <3 independent data sets, and thus no meta-analysis was performed in our field synopsis. The most significantly associated SNPs per locus were rs1129038 in HERC2 (on chromosome 15q13.1, OR = 0.69, \( P = 2.58 \times 10^{-8} \)) and rs17119490 located in an intergenic region on chromosome 10q25.1 (OR = 8.4, \( P = 7.21 \times 10^{-12} \); Supplementary Table S3 online).

DISCUSSION
The MelGene database represents a comprehensive, systematically updated, online synopsis of genetic association studies in CM (Chatzinasiou et al., 2011). This freely available database is curated by experienced researchers to summarize the volume of the evidence of association between genetic variants and CM risk. To our knowledge, this is one of the few field synopsis databases that is continuously updated since its launch 37 months ago (Belbasis et al., 2014). Up to now more than 1,000 SNPs across almost 300 genes implicated in melanoma risk can be accessed and synthesized wherever possible. This reflects the value of meta-analysis and the accumulation of large sample sizes in the field of genetics in minimizing the false-positive signals. Gathering empirical evidence will allow greater insight in the assessment of
cumulative evidence on genetic associations. Among others, it may serve as an ideal resource for selecting the most prominent risk variants to include in future risk prediction models in an effort to improve risk stratification and cost-effectiveness of screening campaigns (Cust et al., 2013). Recent evidence supports the additive effect of common genetic variants in predicting melanoma risk and their potential use in enhancing the value of risk prediction models that contain standardized phenotypic risk factors (Fang et al., 2013; Stefanaki et al., 2013).

Our main meta-analysis yielded genome-wide significant evidence for association with CM for 10 loci, including 3 loci (ATM, FTO, and CASP8; Barrett et al., 2011; Iles et al., 2013; Pena-Chilet et al., 2013) that were not included in our previous meta-analysis (Chatzinasiou et al., 2011). Moreover, four loci (i.e., MITF, CCND1, PLA2G6, and MX2) showed suggestive but sub-genome-wide significant association with CM in our main analyses. The supplementary meta-analyses confirmed one additional independent locus that showed genome-wide significant association with CM (rs7412746 on chromosome 1q21; Amos et al., 2011), which was not included in our previous meta-analyses. Even though a number of independent signals were found to have a strong association with melanoma in certain loci (Table 1), some of

### Table 1. Genetic variants associated with cutaneous melanoma after meta-analyses of at least four independent data sets (main meta-analysis)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Position</th>
<th>Nearest gene</th>
<th>SNP</th>
<th>Data sets (n)</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>Amount of evidence</th>
<th>Venice validation grade</th>
<th>t²</th>
<th>Venice bias grade</th>
<th>Bias reason</th>
<th>Venice overall grade</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>1322087</td>
<td>CLPTM1L</td>
<td>rs401681</td>
<td>10</td>
<td>1.19 (1.12–1.26)</td>
<td>1.42 × 10⁻⁰⁸</td>
<td>A</td>
<td>B</td>
<td>45</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>12672097</td>
<td>TYRPI</td>
<td>rs1408799</td>
<td>7</td>
<td>0.91 (0.84–0.98)</td>
<td>0.012</td>
<td>A</td>
<td>B</td>
<td>31.9</td>
<td>C</td>
<td>Low OR</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>21816528</td>
<td>(MTAP)</td>
<td>rs7023329</td>
<td>6</td>
<td>0.83 (0.80–0.86)</td>
<td>1.11 × 10⁻²⁵</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>21968159</td>
<td>CDKN2A</td>
<td>rs3088440</td>
<td>8</td>
<td>1.27 (1.10–1.46)</td>
<td>0.0009</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>C</td>
<td>F, HWE</td>
<td></td>
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<tr>
<td>15</td>
<td>28230318</td>
<td>OCA2</td>
<td>rs1800407</td>
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<td>1.38 (1.09–1.74)</td>
<td>0.007</td>
<td>B</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>33576989</td>
<td>MYH7B</td>
<td>rs1885120</td>
<td>5</td>
<td>1.55 (1.41–1.71)</td>
<td>1.60 × 10⁻¹⁸</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>33951693</td>
<td>SLC45A2</td>
<td>rs16891982</td>
<td>10</td>
<td>0.42 (0.35–0.50)</td>
<td>1.47 × 10⁻²³</td>
<td>A</td>
<td>A</td>
<td>43</td>
<td>A</td>
<td>A</td>
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</tr>
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<td>22</td>
<td>38545619</td>
<td>PLA2G6</td>
<td>rs6001027</td>
<td>5</td>
<td>0.86 (0.80–0.91)</td>
<td>7.54 × 10⁻⁷</td>
<td>A</td>
<td>B</td>
<td>48.3</td>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>42746081</td>
<td>MX2</td>
<td>rs45430</td>
<td>5</td>
<td>0.89 (0.85–0.94)</td>
<td>1.07 × 10⁻⁵</td>
<td>A</td>
<td>B</td>
<td>26</td>
<td>C</td>
<td>Low OR</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>48239835</td>
<td>VDR</td>
<td>rs1544410</td>
<td>7</td>
<td>0.90 (0.83–0.96)</td>
<td>0.004</td>
<td>A</td>
<td>A</td>
<td>10.8</td>
<td>C</td>
<td>Low OR</td>
<td></td>
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<tr>
<td>16</td>
<td>54114824</td>
<td>FTO</td>
<td>rs16953002</td>
<td>14</td>
<td>1.16 (1.11–1.20)</td>
<td>3.6 × 10⁻¹²</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>69382767</td>
<td>CCND1</td>
<td>rs11263498</td>
<td>4</td>
<td>1.11 (1.05–1.18)</td>
<td>4.6 × 10⁻⁴</td>
<td>A</td>
<td>B</td>
<td>45</td>
<td>C</td>
<td>Low OR</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70014091</td>
<td>MITF</td>
<td>rs149617956</td>
<td>4</td>
<td>2.34 (1.64–3.34)</td>
<td>2.52 × 10⁻⁶</td>
<td>B</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>89017961</td>
<td>TYR</td>
<td>rs1126809</td>
<td>9</td>
<td>1.20 (1.14–1.26)</td>
<td>7.88 × 10⁻¹²</td>
<td>A</td>
<td>A</td>
<td>11.7</td>
<td>A</td>
<td>A</td>
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</tr>
<tr>
<td>16</td>
<td>89755903</td>
<td>CDK10</td>
<td>rs258322</td>
<td>4</td>
<td>1.64 (1.44–1.86)</td>
<td>3.08 × 10⁻¹⁰</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>A</td>
<td></td>
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<tr>
<td>16</td>
<td>90066936</td>
<td>AFG3L1</td>
<td>rs4785763</td>
<td>5</td>
<td>1.35 (1.27–1.44)</td>
<td>1.05 × 10⁻²⁰</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>103528002</td>
<td>XPG</td>
<td>rs17655</td>
<td>4</td>
<td>0.91 (0.82–1.00)</td>
<td>0.043</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>C</td>
<td>Low OR</td>
<td></td>
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<tr>
<td>11</td>
<td>108175462</td>
<td>ATM</td>
<td>rs1801516</td>
<td>5</td>
<td>0.84 (0.79–0.89)</td>
<td>1.49 × 10⁻⁹</td>
<td>A</td>
<td>A</td>
<td>0</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>202143928</td>
<td>CASP8</td>
<td>rs10931936</td>
<td>4</td>
<td>1.15 (1.09–1.21)</td>
<td>2.7 × 10⁻⁸</td>
<td>A</td>
<td>A</td>
<td>8.5</td>
<td>A</td>
<td>A</td>
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<td>1</td>
<td>226564691</td>
<td>PARP1</td>
<td>rs3219090</td>
<td>4</td>
<td>0.86 (0.78–0.93)</td>
<td>0.0003858</td>
<td>A</td>
<td>C</td>
<td>56.2</td>
<td>A</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; F, statistical significance lost excluding first study; HWE, statistical significance lost excluding Hardy–Weinberg equilibrium-violating studies in control subjects; low OR, an OR <1.15; MAF, minor allele frequency in controls when combining all eligible data sets; NA, not applicable; No. of minor alleles, number of minor alleles in patients and control subjects combined across all included data sets; OR, odds ratio.

1Nearest gene denotes the gene in the respective locus or the most proximal gene in the respective locus if the SNP itself does not map into a gene region. It should be noted that these genes are not necessarily the genes that are functionally affected by the genetic association finding in this locus. The location is on the basis of the Human Genome Build hg19.

2Allelic odds ratios, 95% confidence intervals, and P-values (two-sided) were calculated by the DerSimonian–Laird random-effects model.

3Each statistically significant meta-analysis result was graded according to the Human Genome Epidemiology Network Venice criteria. Venice grading: A, grade A (strong epidemiological credibility); B, grade B (moderate epidemiological credibility); and C, grade C (weak epidemiological credibility).

4Criterion does not apply to meta-analysis results if it remained genome-wide significant (P<5 × 10⁻⁸) after exclusion of the initial study.

If multiple polymorphisms showed a statistically significant association in the same locus, only the variant with the best Venice grading is listed here. When the Venice grading yielded equivalent scores, the variant with the smallest P is listed.
isms in genes controlling melanogenesis such as MC1R, SLC45A2, and TYR or loci encompassing relevant genes (CDK10/AFG3L1/MC1R, MYH7B/PIGU/ASIP), assessed primarily by candidate-gene studies and later confirmed by CM GWAS (Brown et al., 2008; Bishop et al., 2009), showed genome-wide significant association with CM risk in our analysis. In addition, genes involved in cell growth and apoptosis, i.e., PLA2G6, tumor suppression, i.e., CDKN2A/MTAP, or telomerase length, i.e., TERT-CLPTM1L, all of which were recognized as nevus-associated genes through GWAS or large-scale association studies (Falchi et al., 2009; Barrett et al., 2011; Law et al., 2012), were supported as genomewide or nominally significantly associated variants with melanoma in our analysis. Recent GWAS have reported on several other putative CM risk loci such as ATM (11q22.3), MX2 (22q22.3), PARP1 (1q42), CASP8 (2q33.1), and CCND1 (11q13.3; Amos et al., 2011; Barrett et al., 2011; Macgregor et al., 2011). All these loci were included in our analysis, yielding nominal significance (MX2, CCND1, and PARP1) or genome-wide significance for association with CM (ATM and CASP8). These genes may operate beyond the two best-characterized clinical phenotypes (nevi and pigmentation) in roles that affect DNA damage repair, cell cycle control, and senescence (Law et al., 2012).

Several of the above listed loci have been functionally investigated or have been implicated in the pathophysiology of cancers other than melanoma. SNP rs149617956, which showed suggestive evidence for association with CM in the MelGene meta-analysis, is a rare germline variant of MITF (Yokoyama et al., 2011). Interestingly, MITF has been implicated in melanoma oncogenesis by regulating several genes that have a role in the development, differentiation, cell-cycle regulation, melanin production, and survival of melanocytes (Bertolotto et al., 2011; Yokoyama et al., 2011; Ghiorzo et al., 2013). The results of our analysis confirm the previously reported association (Bertolotto et al., 2011; Yokoyama et al., 2011). Functional studies have shown an impaired sumoylation of the MITF protein encoded by the minor allele of rs149617956 and a differential regulation of several of its targets, resulting in a gain-of-function role in tumorigenesis (Yokoyama et al., 2009; Bertolotto et al., 2011). To our knowledge, our study provides the first meta-analysis of this variant, strengthening its statistical association with melanoma risk after including a larger number of available data sets.

A recent GWAS of the GenoMEL Consortium (Barrett et al., 2011) reported association of rs1801516 in ATM, a DNA damage response gene, with melanoma without an effect on pigmentation or nevus phenotypes. The same variant also modifies the risk for ionizing radiation-induced or sporadic papillary thyroid carcinoma in addition to BRCA1 and p53 variants (Akulevich et al., 2009; Wojcicka et al., 2014). Although association of ATM and risk of breast cancer has been reported as well, this was not confirmed in a recent meta-analysis (Gao et al., 2010). CASP8 is a member of the caspase family that induces apoptotic cell death mediated by FAS and FASLG (Li et al., 2008). Although the variant that showed genome-wide significant association with CM (rs10931936) has not been investigated for its functional relevance to melanoma, other variants in CASP8, including a nonsynonymous, putatively functional polymorphism (rs1045485) encoding a D302H substitution, have been associated with a variety of cancers—i.e., lung, colon, and breast cancer (Yin et al., 2010). FTO has been associated with multiple traits, including end-stage-renal disease, myocardial infarction, osteoarthritis, and endometrial cancer, most of which appear to be related to body mass index and obesity. Rs16953002, located in intron 8 (in contrast to the known body mass index-related variants that are located in intron 1) showed genome-wide significant association with melanoma independently of body mass index, suggesting a broader function of FTO beyond body mass index and obesity (Iles et al., 2013).

Our approach in curating and analyzing the genetic association data has certain limitations. Missing data from original studies, i.e., genotype summary data, effect size estimates, or direction of effect, were commonly encountered problems that were partially resolved by either comparing with reference panels (1,000 Genomes, HapMap) or, in more rare cases, contacting the authors of the original studies. Moreover, even in studies in which the population comprises people of the same descent, we cannot exclude a potential ethnic substructure that can introduce heterogeneity (Evangelou and Ioannidis, 2013). Other issues included inherent methodological errors in the original studies such as errors in genotyping or sequencing, discrepancies in defining allele names and difficulties in identifying data set overlaps. In addition, our inclusion strategies were primarily based on study-level summary data, precluding the possibility of more refined analyses—i.e., inclusion of potential confounders, analysis of gene–gene or gene–environment interactions, and de novo imputation of genotypes. Moreover, our approach excludes any dominant mutations or rare allele variants.

In summary, we present herein the update of our on-going effort to systematically annotate and analyze all published genetic association studies in melanoma. The online version of Melgene (www.melgene.org) has been embedded with a number of technical refinements that enable improved functionalities (Athanasiadis et al., 2014). Our freely available, user-friendly database serves as a useful model of quantitatively and qualitatively assessing the impact of genetic variation in melanoma risk, and it can also link emerging data on genetic associations with other sources of information on the biology of genes, gene–gene, or gene–environment interactions.
MATERIALS AND METHODS
Literature searches, data extraction, and statistical analyses were performed as previously described and are summarized here only briefly (Chatzinasiou et al., 2011).

Literature search of eligible studies and data extraction
To identify potential association studies eligible for inclusion in MelGene, we searched the PubMed database using the terms “melanoma AND associat***”. In addition we searched the Human Genome and Epidemiology Network Navigator (http://hugeneavigator.net/HuGENavigator) and the Melanoma Molecular Maps Project (http://www.mmmmp.org/MMMP) for additional publications. The last literature search was conducted on 31 August 2013. Studies included in MelGene had to meet the following criteria: (1) to evaluate the association between CM and one or more polymorphisms in a case-control setting, (2) to be published in a peer reviewed journal, and (3) to be published in English.

We excluded studies without a healthy control group, studies of patients with melanoma other than CM (such as uveal melanoma), and studies that examined highly penetrant mutations that were only presented in the patient and not in the control group. Family-based studies and studies of polymorphisms in mtDNA were included in the qualitative overview of the database but excluded from the statistical analyses. Two loci were considered independent if $r^2 < 0.3$ using 1KG project for populations of European descent (Genomes Project C et al., 2012). In those cases where linkage disequilibrium could not be calculated, we consider an “independent locus” as the most significant meta-analysis result in a region ± 1 Mb.

From all eligible studies we extracted the first author’s name, the year of publication, demographic details of the analyzed data sets, names of polymorphisms analyzed, and the corresponding genotype counts in cases and controls where available, or, alternatively, the additive/allelic OR and standard errors (95% confidence intervals).

Both for candidate-gene studies and GWAS overlapping populations, we included only one study in the respective meta-analyses. Whenever all data were available, the study with the largest sample size was included. In cases where overlapping studies had the exact same population, the first study was included in meta-analyses.

Database
Data of the updated analysis of our field synopsis have been deposited to a dedicated online publicly available central repository, the MelGene database (www.melgene.org). To facilitate the use of the presented data, we have updated the front end of the database and improved its functionalities including tools for automatic generation of random-effects meta-analysis plots in high resolution, summary OR, and heterogeneity calculations, as well as tools suitable for network analysis of the data.

Statistical analysis
Summary ORs and 95% confidence intervals were calculated for each eligible polymorphism from study-specific additive/allelic ORs on the basis of the DerSimonian and Laird (1986) random-effects model. Heterogeneity was assessed using the $I^2$ metric. Heterogeneity is considered as low, moderate, high, and very high for values between 0 and 24%, 25 and 49%, 50 and 74%, and >75%, respectively. In addition to the main meta-analyses including all available data sets, a number of sensitivity analyses were performed—i.e., excluding the initial study and data sets violating Hardy–Weinberg equilibrium in the control group ($P<0.05$). Nominal statistical significance was defined as $P<0.05$ and genome-wide statistical significance as $P<5 \times 10^{-8}$. All statistical tests were two-sided.

Assessment of epidemiologic credibility
Assessments of the epidemiologic credibility of nominally significant meta-analysis results were performed using the Venice Criteria, a grading score developed by the Human Genome Epidemiology Network (Ioannidis et al., 2008), as amended also to accommodate GWAS (Khoury et al., 2009). This procedure has been described previously (Chatzinasiou et al., 2011). Briefly, this grading score assesses the amount of evidence by counting the number of minor alleles, the consistency of replication by assessing the heterogeneity using the $I^2$ metric, and the protection from bias by assessing various sources of potential bias, including small-study effects, deviation from Hardy–Weinberg equilibrium, or loss of statistically significant association ($P<0.05$) on exclusion of the initial study. As a result, based on the previous assessments, the overall epidemiological credibility of a finding is either “strong” (grade “A”), “moderate” (grade “B”), or “weak” (grade “C”). Genome-wide significant results are considered to have strong credibility, regardless of any other features.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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