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 ($P<5\times10^{-8}$) 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 $5\times10^{-8}<P<1\times10^{-3}$. 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

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Abbreviations: CM, cutaneous melanoma; GWS, genome-wide significant; GWAS, genome-wide association study; OR, odd ratio; SNP, single-nucleotide polymorphism

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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 *MC1R* (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 ($P<5\times10^{-8}$). 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), rs1801516 in *ATM* (11q22.3), rs4785763 in *AFG3L1P* (16q24.3), rs258322 in *CDK10* (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 ($5\times10^{-8}<P<0.05$). Four loci showed strong but sub-genome-wide significant evidence for association (i.e., $5\times10^{-8}<P<1\times10^{-3}$): rs149617956 in *MITF* (3p13, OR = 2.34, $P=2.5\times10^{-6}$), rs11263498 in *CCND1* (11q13.3, OR = 1.11, $P=4.6\times10^{-4}$), rs6001027 in *PLA2G6* (22q13.1, OR = 0.86, $P=7.54\times10^{-7}$), and rs45430 in *MX2* (21q22.3; OR = 0.89, $P=1.07\times10^{-5}$). 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 ($I^2=0$, 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, $P=9\times10^{-11}$; 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, $P=2.58\times10^{-8}$) and rs17119490 located in an intergenic region on chromosome 10q25.1 (OR = 8.4, $P=7.21\times10^{-12}$; 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

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

Chromosome	Position	Nearest gene[1]	SNP	Data sets (*n*)	OR (95% CI)	*P*	Amount of evidence	Venice validation grade	I^2	Venice bias grade	Bias reason	Venice overall grade[3]
5	1322087	CLPTM1L	rs401681	10	1.19 (1.12–1.26)	1.42×10^{-08}	A	B	45	A		A
9	12672097	TYRP1	rs1408799	7	0.91 (0.84–0.98)	0.012	A	B	31.9	C	Low OR	C
9	21816528	(MTAP)	rs7023329	6	0.83 (0.80–0.86)	1.11×10^{-25}	A	A	0	A		A
9	21968159	CDKN2A	rs3088440	8	1.27 (1.10–1.46)	0.0009	A	A	0	C	F, HWE	C
15	28230318	OCA2	rs1800407	4	1.38 (1.09–1.74)	0.007	B	A	0	A		B
20	33576989	MYH7B	rs1885120	5	1.55 (1.41–1.71)	1.60×10^{-18}	A	A	0	A		A
5	33951693	SLC45A2	rs16891982	10	0.42 (0.35–0.50)	1.47×10^{-23}	A	A[4]	43	A[4]		A
22	38545619	PLA2G6	rs6001027	5	0.86 (0.80–0.91)	7.54×10^{-7}	A	B	48.3	A		B
21	42746081	MX2	rs45430	5	0.89 (0.85–0.94)	1.07×10^{-5}	A	B	26	C	Low OR	C
12	48239835	VDR	rs1544410	7	0.90 (0.83–0.96)	0.004	A	A	10.8	C	Low OR	C
16	54114824	FTO	rs16953002	14	1.16 (1.11–1.20)	3.6×10^{-12}	A	A	0	A		A
11	69382767	CCND1	rs11263498	4	1.11 (1.05–1.18)	4.6×10^{-4}	A	B	45	C	Low OR	C
3	70014091	MITF	rs149617956	4	2.34 (1.64–3.34)	2.52×10^{-6}	B	A	0	A		B
11	89017961	TYR	rs1126809	9	1.20 (1.14–1.26)	7.88×10^{-12}	A	A	11.7	A		A
16	89755903	CDK10	rs258322	4	1.64 (1.44–1.86)	3.98×10^{-14}	A	A	0	A		A
16	90066936	AFG3L1	rs4785763	4	1.35 (1.27–1.44)	1.05×10^{-20}	A	A	0	A		A
13	103528002	XPG	rs17655	4	0.91 (0.82–1.00)	0.043	A	A	0	C	Low OR	C
11	108175462	ATM	rs1801516	5	0.84 (0.79–0.89)	1.49×10^{-9}	A	A	0	A		A
2	202143928	CASP8	rs10931936	4	1.15 (1.09–1.21)	2.7×10^{-8}	A	A	8.5	A		A
1	226564691	PARP1	rs3219090	4	0.86 (0.78–0.93)	0.0003858	A	C	56.2	A		C

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.

[1]''Nearest 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.

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

[3]Each 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 (modest epidemiological credibility); and C, grade C (weak epidemiological credibility).

[4]Criterion does not apply to meta-analysis results if it remained genome-wide significant ($P<5 \times 10^{-8}$) 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.

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

these genes may not represent true susceptibility variants but may simply tag other genes within the same locus that have a more biologically plausible association with melanoma. For example, AFG3L1P and CDK10 genes on chromosome 16 and MHY7B on chromosome 20 most likely tag the well-known pigmentation genes of MC1R and ASIP, respectively (Bishop *et al.*, 2009); e.g., the polymorphism rs258322 in CDK10 (16q24.3) is in linkage disequilibrium with the red hair allele of MC1R rs1805007, derived from data from the 1,000 Genomes (1KG) project ($r^2 = 0.58$).

Our results may contribute to a more comprehensive view of the genetic architecture of CM predisposition. Polymorphisms in genes controlling melanogenesis such as *MC1R*, *SLC45A2*, and *TYR* or loci encompassing relevant genes (CDK10/*AFG3LIP*/*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 genome-wide 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 confirms 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://hugenavigator.net/HuGENavigator) and the Melanoma Molecular Maps Project (http://www.mmmp.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 $P<5\times10^{-8}$. 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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