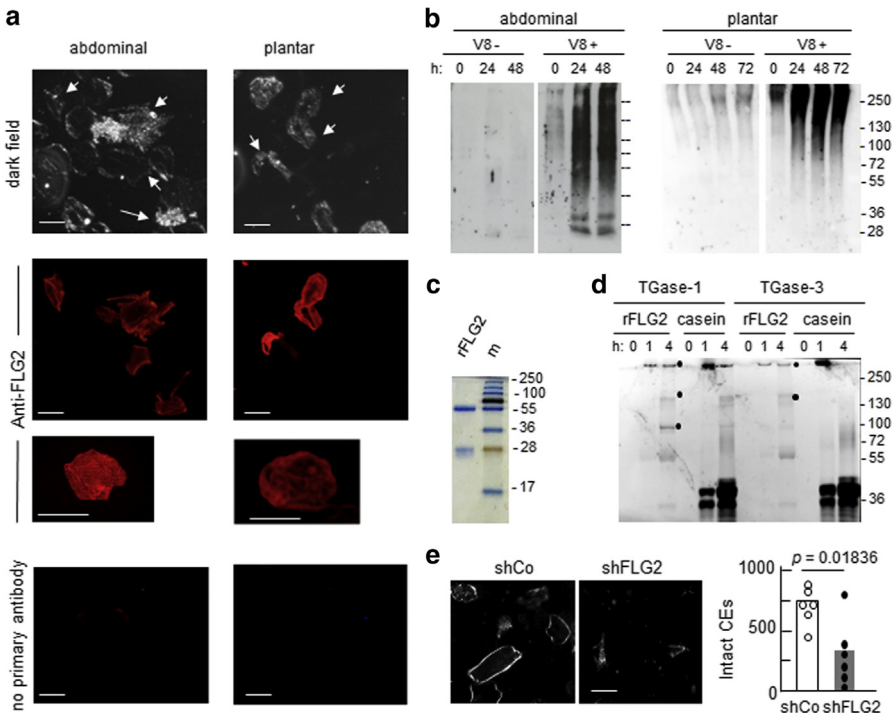

Figure 2. FLG2 spacer domain is cross-linked to CEs. (**a**) CEs purified from abdominal and plantar human skin were observed in dark field and immunodetected with the anti-FLG2 antibody (red) or without primary antibody. Arrows in the upper panels indicate immunolabeled envelopes. A typical immunodetected envelope from each anatomical site is enlarged in the middle panels. Scale bar = 50 μm. (**b**) The CEs were analyzed by immunoblotting with the same antibody after incubations without ($V8^-$) or with ($V8^+$) V8 protease for 0–72 hours, as indicated. (**c**) The recombinant FLG2 spacer domain (rFLG2) was Coomassie blue stained after separation by SDS gel electrophoresis. Although its theoretical molecular mass is 15.66 kDa, two bands around 27 and 55 kDa were observed. (**d**) Casein, as a control, and rFLG2 were incubated with either TGase-1 or TGase-3 for 0, 1 and 4 hours in the presence of $CaCl_2$ and monodansylcadaverine. Samples were separated by SDS gel electrophoresis, and incorporation of monodansylcadaverine was revealed by UV illumination. •, high-molecular-weight cross-linked complexes. The position of molecular mass markers (m) is indicated on the right. (**e**) Reconstructed human epidermis were produced with keratinocytes from one donor and transduced with either control small hairpin RNA (shCo) or *FLG2*-specific small hairpin RNA (shFLG2). In each condition, CEs were purified from four pooled epidermis. The same amount of CEs were then sonicated for 5 seconds (n = 6 independent experiments), and the remaining intact envelopes were observed under a dark field microscope and quantified. CE, cornified envelope; TGase, transglutaminase.

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A Transethnic Mendelian Randomization Study Identifies Causality of Obesity on Risk of Psoriasis

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Abbreviations: BMI, body mass index; GWAS, genome-wide association study; IVW, inverse variance-weighted; MR, Mendelian randomization; SNP, single nucleotide polymorphism

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TO THE EDITOR

Psoriasis is a chronic disorder characterized by cutaneous and systemic manifestations. Epidemiological studies have reported increased comorbidity of psoriasis with numerous complex

Table 1. Results of the transethnic MR analyses inferring causality of the clinical metabolic measurements on psoriasis

Trait	MR Method	MR Result in Europeans		MR Result in Japanese		Meta-Analysis
		Beta (SE)	*P*	Beta (SE)	*P*	*P*
Body mass index	Inverse variance weighted	0.464 (0.112)	3.1×10^{-5}	1.275 (0.472)	0.0069	1.2×10^{-6}
	MR-Egger	0.697 (0.262)	0.0093	1.499 (1.342)	0.27	0.0088
Triglyceride	Inverse variance weighted	0.085 (0.088)	0.33	0.139 (0.369)	0.71	0.34
	MR-Egger	−0.064 (0.129)	0.62	0.748 (0.561)	0.19	0.56
Total cholesterol	Inverse variance weighted	0.054 (0.063)	0.39	−0.595 (0.476)	0.21	0.78
	MR-Egger	0.126 (0.115)	0.28	−0.663 (0.900)	0.47	0.80
HDL cholesterol	Inverse variance weighted	−0.026 (0.072)	0.72	0.190 (0.318)	0.55	0.87
	MR-Egger	0.024 (0.109)	0.82	−0.048 (0.509)	0.93	0.92
LDL cholesterol	Inverse variance weighted	0.051 (0.054)	0.35	−0.300 (0.385)	0.44	0.91
	MR-Egger	0.180 (0.088)	0.046	−0.134 (0.535)	0.80	0.22
Blood sugar	Inverse variance weighted	−0.537 (0.269)	0.046	0.235 (0.671)	0.73	0.24
	MR-Egger	−0.685 (0.943)	0.47	1.940 (2.550)	0.46	0.99
HbA1c	Inverse variance weighted	−0.143 (0.273)	0.60	−0.289 (0.417)	0.49	0.39
	MR-Egger	0.064 (0.552)	0.91	1.811 (1.661)	0.29	0.41
Systolic blood pressure	Inverse variance weighted	0.006 (0.010)	0.54	1.080 (0.781)	0.17	0.16
	MR-Egger	0.048 (0.033)	0.16	3.818 (2.823)	0.19	0.055
Diastolic blood pressure	Inverse variance weighted	0.017 (0.017)	0.31	1.010 (0.963)	0.29	0.14
	MR-Egger	0.099 (0.058)	0.10	1.560 (3.043)	0.62	0.13

Abbreviations: HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MR, Mendelian randomization; SE, standard error.

diseases such as metabolic clinical measurements (Greb et al., 2016; Naito and Imafuku, 2016). However, interpretation of the comorbidity remains controversial to date, because causal inference between correlated phenotypes is difficult when depending solely on epidemiological studies. Identification of causal inference between correlated phenotypes has significant clinical impacts, because modification of the causal phenotypes could benefit treatment of the outcome phenotypes. Drugs indicated by the causal phenotypes could also be promising targets of drug repositioning for the outcome phenotypes (Holmes et al., 2017). Therefore, alternative approaches to strengthen causal inference on psoriasis are warranted.

An approach becoming popular for this purpose is use of genetic data (Pingault et al., 2018). Genetically determined phenotype profiles are robust to confounding factors acquired during a lifetime, which could be interpreted as ideal randomization of subjects. Mendelian randomization (MR) is an approach to infer causal inference between phenotypes using the genome-wide association study (GWAS) results (Holmes et al., 2017; Hemani et al., 2018). Because of (i) achievement of large-scale GWASs of a variety of human phenotypes with public data deposit and (ii) development of MR analytical methods that robustly infer causality, such as MR-Egger (Burgess and Thompson, 2017),

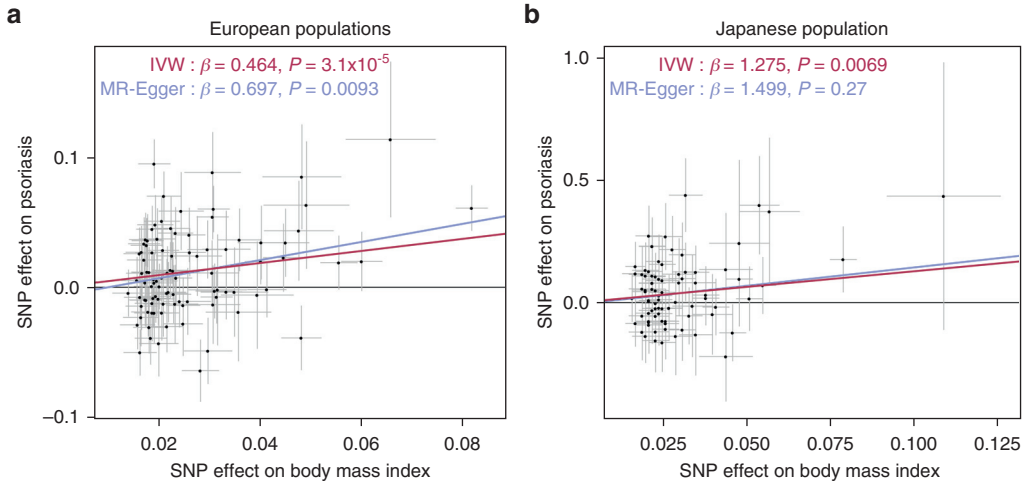

Figure 1. Regression plots of the BMI-associated variants on psoriasis risk. Dots represent the BMI-associated SNPs plotted along with effect size estimates on BMI (x-axis) and psoriasis risk (y-axis) with 95% confidence intervals in (**a**) the European populations and (**b**) the Japanese population. Regression lines obtained from the MR analyses are plotted in red (by IVW) and blue (by MR-Egger). BMI, body mass index; IVW, inverse variance weighted; MR, Mendelian randomization; SNP, single nucleotide polymorphism.

MR is now one of the best approaches to infer causality. Generally, the largest available GWAS result within a single ancestry is used for an MR analysis to afford robust conclusions. Thus, confirmation of the MR analysis results requires additional validation using GWAS with independent ancestry.

Here, we conducted a transethnic MR analysis to estimate causal inference on psoriasis. We obtained the genome-wide summary statistics of the previously reported psoriasis GWAS of European populations (13,229 case and 21,543 control individuals) (Tsoi et al., 2017) and Japanese population (282 case and 426 control individuals) (Hirata et al., 2018) (see Supplementary Table S1 online). Both of the psoriasis GWASs were conducted by applying whole-genome sequencing–based genotype imputation, which yielded high coverage of the genome-wide variants suitable for the MR analysis (>6,000,000 variants). As for the European GWAS, the participants in the cohort collected by 23andMe (Mountain View, CA) were excluded because of their policies on summary data sharing. We thus reconducted the GWAS meta-analysis after sample exclusion for our MR analyses. We admit that the sample size of the Japanese GWAS was relatively smaller, which warrants further accumulation of participants.

We focused on metabolic clinical measurements as exposure phenotypes, for which the large-scale GWAS results have been released in both populations. We selected nine measurements: obesity (body mass index [BMI]); levels of triglycerides, total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, blood sugar, and hemoglobin A1c; and systolic and diastolic blood pressure (on average 149,958 subjects per trait) (see Supplementary Table S2 online) (Akiyama et al., 2017; Ehret et al., 2011; Kanai et al., 2018; Locke et al., 2015; Scott et al., 2012; Wheeler et al., 2017; Willer et al., 2013). After selection of the lead variants (or the proxy single nucleotide polymorphisms [SNPs] in linkage disequilibrium of $r^2 \geq 0.5$ in the corresponding 1000 Genomes Project phase1v3 populations) at the loci with genome-wide significance threshold ($P < 5.0 \times 10^{-8}$) and exclusion of the highly pleiotropic locus of the major histocompatibility complex region, on average 41.3 loci per trait were obtained.

We adopted two-sample MR, one of the MR analysis approaches that handles summary statistics obtained from separate studies. In addition to the typical method of inverse variance weighted (IVW), we adopted MR analysis based on Egger regression (i.e., MR-Egger), which is statistically less powerful but more robust to bias caused by directional pleiotropy (Burgess and Thompson, 2017). We used the MR-Base platform implemented as a package of R statistical software (R Core Development Team, Vienna, Austria) (Hemani et al., 2018).

For Europeans, significant causality of genetically increased BMI on risk of psoriasis was estimated ($\beta = 0.464$ and $P = 3.1 \times 10^{-5}$ in IVW, and $\beta = 0.697$ and $P = 0.0093$ in MR-Egger) (Table 1 and Figure 1). For Japanese, significant causality of BMI on psoriasis risk was also observed in the IVW analysis with a concordant directional effect ($\beta = 1.275$ and $P = 0.0069$). Although the MR-Egger result was not significant ($\beta = 1.499$ and $P = 0.27$), the effect size estimate was larger than that of IVW. Because phenotype normalization methods were different among the original GWAS, direct comparison of the estimated causal effect sizes of the BMI-associated SNPs on psoriasis between the studies was difficult. We assessed potential bias in the results of MR analyses, mostly caused by SNP pleiotropy, by applying a set of sensitivity analyses including heterogeneity test, leave-one-out analysis, and funnel plots implemented in the MR-Base platform, but we did not find existence of apparent bias (see Supplementary Figure S1 online). We applied the reverse MR analysis inferring causality of psoriasis on BMI, but we did not observe significant causality ($P > 0.75$) (see Supplementary Figure S2 online).

We then conducted a transethnic meta-analysis of the MR results, using weighted summation of z-scores considering directional concordance of the causal effect estimates. As expected, significant causality of BMI on risk of psoriasis was observed ($P = 1.2 \times 10^{-6}$ in IVW, and $P = 0.0088$ in MR-Egger). Although suggestive relationships of LDL cholesterol and blood sugar were observed in Europeans ($P < 0.05$ in IVW or MR-Egger), these associations were not replicated in Japanese, and transethnic MR analyses did not indicate significant causality of these traits ($P > 0.22$).

By using large-scale GWAS results of psoriasis in European and Japanese populations, our transethnic MR analyses identified a causal link for obesity on risk of psoriasis. This study has value as one of the initial successful examples of transethnic MR analysis. Epidemiological studies have pointed to a link between higher BMI and increased incidence or severity of psoriasis (Greb et al., 2016; Naito and Imafuku, 2016), with which our MR analysis results were concordant. Moreover, because our study validated causality (i.e., directional link) of obesity on psoriasis, interventional improvement of obesity itself could be a promising treatment strategy toward better management of psoriasis. Further application of the MR analysis of psoriasis to a wider range of phenotypes, as well as validations in additional ethnicities, is warranted.

ORCID

James T. Elder: http://orcid.org/0000-0003-4215-3294

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Kotaro Ogawa[1,2], Philip E Stuart[3], Lam C. Tsoi[3,4,5], Ken Suzuki[1], Rajan P. Nair[3], Hideki Mochizuki[2], James T. Elder[3,6] and Yukinori Okada[1,7,*]

[1]Department of Statistical Genetics, Osaka University Graduate School of Medicine, Suita, Japan; [2]Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan; [3]Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, USA; [4]Department of Biostatistics, Center for Statistical Genetics,

University of Michigan, Ann Arbor, Michigan, USA; [5]Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA; [6]Ann Arbor Veterans Affairs Hospital, Ann Arbor, Michigan, USA; and [7]Laboratory of Statistical Immunology, Immunology Frontier Research Center (WPI-IFReC), Osaka University, Suita, Japan
**Corresponding author e-mail: yokada@sg.med.osaka-u.ac.jp*

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.11.023.

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IL-36 Signaling Is Essential for Psoriatic Inflammation through the Augmentation of Innate Immune Responses

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TO THE EDITOR

IL-36 cytokines are composed of three agonists, namely IL-36α, IL-36β, and IL-36γ, and a natural antagonist, IL-36Ra (Sims and Smith, 2010). IL-36 cytokines are abundantly expressed by the skin and other epithelial tissues, whereas the IL-36 receptor (IL-36R) is expressed by skin and immune cells, including dendritic cells (DCs) (Gabay and Towne, 2015; Vigne et al., 2011). Earlier studies have demonstrated that IL-36 cytokines play important roles in the development of psoriasiform inflammation by enhancing the function of T helper type 17 cytokines (Carrier et al., 2011; Tortola et al., 2012). Indeed, IL-36 signaling–regulated genes in keratinocytes (KCs) are largely shared with those observed in psoriatic lesions, and they form interconnected feedback loops that potentiate IL-17 signaling and leukocyte chemotaxis (Carrier et al., 2011; Mahil et al., 2017), although the details remain largely unknown.

Psoriatic lesions demonstrated increased IL-36α, IL36-γ, IL-36Ra, but not IL-36β, as reported previously (see Supplementary Figure S1a online). Similarly, all of the IL-36 family members were upregulated in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)–induced psoriatic lesions in K5.Stat3C model (see Supplementary Figure S1b), which required the IL-23/Th17 pathway (Nakajima et al., 2011).

To explore the role of IL-36 signaling in KCs, we examined psoriasis-related gene expression in response to exogenous IL-36 in between control and IL-36R$^{-/-}$ (knockout [KO]) KCs derived from IL-36R KO mice. Following IL-36α stimulation, wild-type KCs expressed increased *Il36a, S100a8, Defb3,* and *Il17c* mRNAs, and also IL-17C protein (Figure 1a). In contrast, the upregulation of those was not observed in IL-36R KO KCs. Similar results were obtained when we used other IL-36 cytokines, namely, IL-36β and IL-36γ (Supplementary Figure S2 online). These results strongly implicated the participation of the IL-36/IL-36R autocrine loop within KCs in psoriasis

Abbreviations: DC, dendritic cell; Ht, heterozygous; IL-36R, IL-36 receptor; KC, keratinocyte; KO, knockout; TPA, 12-O-tetradecanoylphorbol-13-acetate

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