ORIGINAL ARTICLE

A Deep Intronic Polymorphism at 9q21.11 Contributes to the Risk of Atopic Dermatitis Through Methylation-Regulated Expression of Tight Junction Protein 2

Lim YYE[1], Sio YY[1], Say Y-H[1,2,3], Reginald K[3], Chew FT[1]

[1]Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore
[2]Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR) Kampar Campus, Kampar, Perak, Malaysia
[3]Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Malaysia

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Abstract

Background: Atopic dermatitis (AD) is a chronic inflammatory itchy skin condition. Genome- and epigenome-wide association studies provide insights into genetic susceptibility and the pathogenesis of potential underlying disease.
Objective: This study sought to functionally characterize an AD-associated single-nucleotide polymorphism (SNP) located deep intronic of the tight junction protein 2 (*TJP2*) gene (9q21.11 locus), identified through a genome-wide association study (GWAS).
Methods: The association between the 9q21.11 locus (rs7872806) and AD was identified through a GWAS of 956 cases and 723 controls. *TJP2* expression in peripheral blood mononuclear cells (PBMCs) was assessed against the rs7872806 genotype. Allele-specific methylation was evaluated at CpG sites 10 kb up- and down-stream of the 9q21.11 locus. The effect of DNA methylation on *TJP2* expression was validated via in vitro methylation and 5-aza-2′-deoxycytidine–induced transcriptional activation studies. Transepidermal water loss (TEWL) measurements were used to determine skin barrier function.
Results: The major allele "G" of rs7872806 was found to increase the risk of AD by 2.64-fold (adjusted *P* value, 2.40×10^{-18}; OR, 0.38) and was associated with increased methylation levels at the cg13920460 site ($P<.001$) and lower *TJP2* expression in PBMCs (Pearson $P=1.09 \times 10^{-6}$, Pearson R, –0.313, $P<.001$). Inhibition of methylation by 5-aza-2′-deoxycytidine increased *TJP2* promoter activity by up to 85%. Elimination of the cg13920460 methylation site increased expression by approximately 25%. The major allele of rs7872806 was also found to be associated with increased TEWL ($P<.001$).
Conclusion: Epigenetic influence at CpG site cg13920460 is associated with rs7872806 located deep intronic at 9q21.11. The SNP confers susceptibility to AD by altering *TJP2* expression and promoting TEWL.

Key words: Atopic dermatitis. Genome-wide association studies. Epigenetics. Methylation. Expression quantitative trait loci. Tight junction protein. Transepidermal water loss. Epidermal hyperplasia.

Resumen

Antecedentes: La dermatitis atópica (DA) es una enfermedad inflamatoria crónica de la piel que produce picor. Los estudios de asociación genómica y epigenética proporcionan información sobre la susceptibilidad genética y la posible patogénesis subyacente de la enfermedad.
Objetivo: Caracterizar funcionalmente un polimorfismo de nucleótido único (SNP) asociado a la DA y localizado en la parte intrónica profunda del gen de la proteína de unión estrecha 2 (*TJP2*) (locus 9q21.11), identificado mediante un estudio de asociación de genoma completo (GWAS).
Métodos: La asociación entre el locus 9q21.11 (rs7872806) y la DA se identificó mediante el estudio GWAS de 956 casos y 723 controles. Se evaluó la expresión de *TJP2* en células mononucleares de sangre periférica (PBMCs) frente a los genotipos rs7872806. Se evaluó la metilación específica del alelo en sitios CpG 10kb corriente arriba y abajo del locus 9q21.11. Los efectos de la metilación del ADN en la expresión de *TJP2* se validaron mediante estudios de metilación *in vitro* y de activación transcripcional inducida por 5-aza-2'-desoxicitidina. Para determinar la función de barrera de la piel, se utilizaron mediciones de pérdida de agua transepidérmica.
Resultados: Se determinó que el alelo principal del rs7872806 aumentaba el riesgo de DA 2,64 veces (p-valor ajustado= $2,40 \times 10^{-18}$, OR= 0,38), asociado con mayores niveles de metilación en el sitio cg13920460 (p< 0,001) y menor expresión de *TJP2* en PBMCs (p de Pearson= $1,09 \times 10^{-6}$, R de Pearson= -0,313, p< 0,001). La inhibición de la metilación mediante 5-aza-2'-desoxicitidina aumentó la actividad promotora de *TJP2* hasta en un 85%. La eliminación del sitio de metilación cg13920460 aumentó la expresión en aproximadamente un 25%. El alelo principal rs7872806 también se asoció con una mayor pérdida de agua transepidérmica (p< 0,001).
Conclusión: La influencia epigenética en el sitio CpG cg13920460 está asociada con rs7872806 localizado intrónicamente en 9q21.11. El SNP confiere susceptibilidad a la DA a través de la alteración de la expresión de *TJP2* y la promoción de la pérdida de agua transepidérmica.

Palabras clave: Dermatitis atópica. Estudios de asociación del genoma completo. Epigenética. Metilación. *Loci* de rasgos cuantitativos de expresión. Proteína de unión estrecha. Pérdida transepidérmica de agua. Hiperplasia epidérmica.

Summary box

- **What do we know about this topic?**
  Atopic dermatitis has a strong genetic component. Previous genome-wide association studies have identified several population-specific single genetic variations. However, lack of functional characterization has led to a gap in understanding heritability.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  This paper demonstrates the involvement of a deep intronic single-nucleotide polymorphism at 9q21.11 (rs7872806) influencing tight junction protein 2 expression through methylation and contributing to onset of atopic dermatitis. The gene identified is a target for epigenetic therapy.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory itchy skin condition with a prevalence of more than 2% worldwide. In Singapore, this allergic disease affects about 13% of the population [1] and decreases the quality of life of both patients and caregivers [2]. It also poses a significant economic burden on Singapore's health care system, and its prevalence is projected to increase [3].

AD is associated with a dysfunction in the skin epidermal barrier and results in increased immunoglobulin E (IgE) levels in response to allergens. The pathogenesis of AD is multifactorial, influenced by both genetic and environmental factors. While the exact etiology and interplay of these factors remain uncertain, genetic predisposition has been shown through family and twin studies. In the European population, the AD concordance rate for monozygotic twins is 0.15 to 0.86, which is about 2 to 3 times higher than the 0.05 to 0.41 observed for dizygotic twins [4]. Individuals with a family history of AD also present with a 2.5-fold increase in susceptibility to AD [5]. Heritability for AD is estimated to be 71% to 84% [6].

As underscored by insights from Armario-Hita et al [7], understanding the genetic foundations of AD will empower personalized therapy, thereby maximizing treatment efficacy. Advancements in genome-wide genotyping arrays have driven the widespread adoption of genome-wide association studies (GWAS) in the quest to identify disease-associated genetic variants. Studies performed in populations of various ethnicities have identified more than 23 AD susceptibility loci to date (GWAS catalog: https://www.ebi.ac.uk/gwas/). Seven risk loci hypothesized to be involved in epidermal barrier function and immune dysregulation were identified in GWAS of individuals of European descent [8,9], 2 were identified in Han Chinese [10], and 2 were identified in the German population [11]. Twelve susceptibility loci were also identified in the Japanese population [12,13]. While some AD susceptibility loci were consistent across populations, a large proportion remained population-specific. This can be attributed to the genetic heterogeneity of the disease between populations of varying ancestry or the presence of background-specific rare single-nucleotide polymorphisms (SNPs) (minor allele frequencies less than 5%) with a very strong disease effect. Hence, current GWAS-identified loci are inadequate for comprehensively describing the pathogenic heritability of AD.

We performed a GWAS for AD in the Chinese population residing in Singapore and identified a new SNP at 9q21.11 (rs7872806) that was strongly associated with the presence of AD. Through the integration of genetic and epigenetic association studies, methylation and expression quantitative trait loci (mQTL, eQTL), in vitro allele-specific luciferase expression effects, and phenotypic evaluations, we attempt to functionally characterize this deep intronic SNP and illustrate its involvement in the pathogenesis of AD.

Materials and Methods

Recruitment of Participants and Description of Study Population

Study participants of self-reported Chinese ethnicity residing in Singapore were recruited from the National University of Singapore (NUS) through various ongoing cross-sectional volunteer recruitment drives initiated in August 2005. Approvals were obtained from the Institutional Review Board of NUS (Reference NUS-07-023, NUS-09-256, NUS-10-445, NUS-13-075, NUS-14-150, and NUS-18-036) in compliance with the Declaration of Helsinki, together with written informed consent. The inclusion criteria were age ≥18 years, Chinese ethnicity, no consumption of antihistamines for at least 3 days prior to the study, and no previous participation by an immediate family member.

The mean (SD) age was 21.46 (4.62) years. Participants were classified as AD cases or controls according to validated guidelines from ISAAC-validated questionnaires [14], the UK Working Party Criteria [15], and the Hanifin and Rajka diagnostic criteria [16]. The clinical symptoms of AD were defined as recurrent flexural itch lasting ≥6 months. A skin prick test (SPT) was performed with house dust mite (HDM) to define atopic status. This showed high sensitivity and specificity in previous studies when compared to the clinical standard based on the presence of allergic comorbidities [17]. The SPT with HDM was the sole method used to assess atopy based on the findings by Andiappan et al [18]. In their study of the Singaporean population, over 80% of IgE-sensitized participants tested positive for HDM-specific IgE. Among those who did not exhibit HDM-specific IgE, only 5% were atopic to other allergens, and, even so, their IgE titers were low. AD cases were thus defined as patients with AD symptoms and a positive atopic status. Atopic and nonatopic controls had a positive and negative atopic status, respectively, with no AD symptoms.

Genotyping and Genome-Wide Association Studies

Genomic DNA was extracted from volunteers' buccal cells using the Axygen® AxyPrep™ Multisource Genomic Miniprep DNA kit and subsequently genotyped using 4 GWAS arrays (Infinium OmniZhongHua-8 v1.3 BeadChip platform; Illumina HumanHap 550 k BeadChip, version 3; InfiniumOmni2-5Exome; and Infinium Global Screening Array). In accordance with the recommendation put forth by Roshyara et al [20], advising imputation before quality control, we proceeded with haplotype phasing and imputation using IMPUTE v2.0 with information from the 1000 Genomes Project phase III Han Chinese in Beijing (CHB) database, which was previously demonstrated to possess high minor allele frequency (MAF) concordance with Singaporean Chinese [21]. Results from all 4 arrays were then combined. The samples underwent quality control. We removed all individuals with a call rate <90%, inconsistent information on sex, and duplicated and related samples. SNPs with call rates <90%, indels, duplicated and monomorphic SNPs, and SNPs that did not pass the Hardy-Weinberg equilibrium filter of $P>.001$ were also removed, yielding 5 215 687 merged SNPs. The detailed data preprocessing pipeline can be found in Supplementary Figure S4.

Non-Chinese individuals were excluded following scrutiny of investigator-administered questionnaire responses, resulting in a cohort comprising 5055 Singaporean individuals of Chinese descent. Given the clinical nature of AD, atopic controls were excluded from further GWAS in order to minimize confounding from other allergic conditions and to more accurately represent individuals with no inherent predisposition to AD. This exclusion also gives us a lower genomic inflation-estimated lambda of 1.04787. In total, we had 1679 unique participants (956 AD cases and 723 nonatopic controls), who were further selected for SNP-disease association analysis (Table S1).

The statistical analysis was performed using PLINK v1.09 and involved logistic regression adjusted for age, sex, and 10 principal components. Manhattan and quantile-quantile plots were drawn using the R v3.6.2 qqman package. PLINK v1.09 was used to identify independent AD association signals and calculate the genomic inflation factors (λ_{GC}), OR, and MAF. SNPs with P values $<5 \times 10^{-8}$ (genome-wide significance threshold for this study) and MAF >5% were further examined. Haploview® version 4.2 was used to calculate the r^2 values, draw linkage disequilibrium (LD) patterns, and determine haplotype frequencies for gene constructs [22]. The methodology used to obtain and select the principal components and the power calculations can be found in the supplementary material.

Transcriptome and Methylation Analysis in Peripheral Blood Mononuclear Cells

Whole blood was collected from 575 Chinese volunteers residing in Singapore and Malaysia from the NUS, Sunway University, or Universiti Tunku Abdul Rahman (UTAR), with approval from the Scientific and Ethical Review Committee (SERC) of UTAR (Ref-code: U/SERC/03/2016), Sunway University Research Ethics Committee (Ref - SUREC 2019/029), and Institutional Review Board at the NUS, Singapore (numbers NUS 07-023, NUS 09-256, and NUS 10-445). Total RNA of peripheral blood mononuclear cells (PBMCs) was extracted following isolation by Ficoll-Hypaque density gradient centrifugation using the E.Z.N.A.® Total RNA kit from Omega Bio-tek Inc. according to the manufacturer's instructions. mRNA was enriched using oligo(dT) beads and the NEBNext Ultra RNA Library Prep Kit prior to sequencing with the Illumina NovaSeq 6000 system. Raw sequences were mapped against the human genome assembly (GRCh37/hg19 version) using TopHat version 2.1.1. Fragments per kb of transcript per million mapped reads (FPKM) were calculated using Cufflinks version 2.2.1, where raw counts were normalized by sequencing depth and gene length. FPKM values were then normalized by quantile. Transcript data (RNAseq) were used for downstream expression quantitative trait loci (eQTL) analysis. The single-cell RNA sequencing (scRNA-seq) dataset "Fig2e-5PBMCs_scRNAseq_matrix.txt" obtained from CIBERSORTx© (Stanford University 2023) was used to create the signature matrix and obtain imputed cell fractions [23,24]. The imputed cell fractions were subsequently applied as covariates in a linear regression analysis to evaluate the impact of cell type heterogeneity on PBMCs in our RNAseq data. Association analysis was conducted using a 2-sample independent t test with unequal variances. Transcript expression was also assessed using publicly available databases, namely, eQTLGen Phase II (available at: https://www.eqtlgen.org/cis-eqtls.html) and GTExPortal version 8 (available at: https://gtexportal.org/).

Genomic DNA was extracted from 233 of the 575 PBMC samples and subjected to bisulphite conversion. The Infinium MethylationEPIC BeadChip Kit (Illumina) was then used to assay the whole methylome. Annotation of the array data was conducted using the manifest file supplied by Illumina (Infinium MethylationEPIC v1.0 B4 Manifest File), which contains information for 865 918 probes (available at: https://sapac.support.illumina.com/downloads/infinium-methylationepic-v1-0-product-files.html). The raw data underwent preprocessing and quantile normalization, followed by the calculation of methylation ß values, utilizing the minfi package within R version 3.6.1. Samples were analyzed concurrently to mitigate potential batch-related variations. Methylation levels at each CpG site 10 kb up- and down-stream flanking the gene were evaluated for association with the genotype via analysis of variance (ANOVA) based on multiple testing corrected by a Bonferroni adjustment or Pearson correlation test. ReFACTor v1.0 was used to generate ReFACTor components, which were subsequently applied to assess the impact of cell type heterogeneity in PBMCs on the results of the methylation analysis through linear regression [25].

Cells and Treatments

Human embryonic kidney 293T cells (HEK293T) were purchased from the American Type Culture Collection (ATCC) and grown in high-glucose Dulbecco modified Eagle medium

Table. List of the 26 AD-Associated SNPs That Pass the GWAS Significance Threshold.[a]

CHR	SNP	*P* Value[b]	BP (GRCh37)	Minor/ major allele	MAF (case/ctrl)	OR (95%CI)[c]	Genes within 50kb of SNP
9	rs7872806	2,40E-18	71772940	A/G	0.086/0.199	0.379 (0.304 - 0.471)	*TJP2*
6	rs62391770	2,19E-17	29664983	T/C	0.072/0.175	0.358 (0.283 - 0.454)	*MOG; ZFP57; HLA-F; HLA-F-AS1*
6	rs148694836	2,41E-17	29665741	G/C	0.072/0.176	0.361 (0.285 - 0.457)	*MOG; ZFP57; HLA-F; HLA-F-AS1*
6	rs56269224	4,36E-17	29669998	G/C	0.073/0.176	0.365 (0.288 - 0.461)	*MOG; ZFP57; HLA-F; HLA-F-AS1; IFITM4P*
21	rs181286089	1,52E-14	27726488	A/G	0.030/0.097	0.273 (0.196 - 0.380)	*CYYR1-AS1*
15	rs1564638	4,39E-14	101895981	G/A	0.059/0.138	0.372 (0.288 - 0.481)	*PCSK6; LOC100507472; PCSK6-AS1*
7	rs34657769	4,68E-12	67366661	A/C	0.029/0.086	0.297 (0.210 - 0.419)	-
16	rs79224535	7,94E-12	84225184	T/C	0.022/0.076	0.263 (0.179 - 0.386)	*HSDL1; DNAAF1; TAF1C; ADAD2; LOC654780; KCNG4*
20	rs2297122	1,03E-11	854807	T/C	0.036/0.094	0.332 (0.242 - 0.456)	*FAM110A; ANGPT4*
9	rs79128444	1,73E-11	31258879	G/A	0.069/0.150	0.441 (0.347 - 0.560)	-
5	rs60507859	6,33E-11	57362947	A/C	0.034/0.097	0.348 (0.254 - 0.478)	*LINC02101*
3	rs111529683	6,01E-10	155001404	A/G	0.046/0.104	0.398 (0.297 - 0.533)	*LINC01487; STRIT1*
2	rs3820864	1,97E-09	169012074	A/C	0.395/0.289	1.583 (1.363 - 1.840)	*STK39*
6	rs241410	2,81E-09	32861651	G/C	0.539/0.429	1.55 (1.341 - 1.791)	*PSMB8; PSMB8-AS1; TAP1; PSMB9; LOC100294145; HLA-DMB*
6	rs3131634	3,07E-09	31477189	A/G	0.025/0.076	0.341 (0.239 - 0.487)	*HCP5; HCG26; MICB-DT; MICB; MCCD1; ATP6V1G2-DDX39B; DDX39B; SNORD117; SNORD84; DDX39B-AS1; ATP6V1G2; NFKBIL1*
11	rs4520594	5,45E-09	11540861	A/G	0.118/0.055	2.325 (1.751 - 3.086)	*GALNT18*
6	rs6938961	7,72E-09	28280297	A/G	0.185/0.109	1.894 (1.525 - 2.353)	*ZSCAN26; PGBD1; ZSCAN31; ZKSCAN3*
6	rs9258051	1,26E-08	29665228	A/G	0.578/0.473	1.552 (1.334 - 1.806)	*MOG; ZFP57; HLA-F; HLA-F-AS1*
15	rs146875672	1,27E-08	44775198	T/C	0.041/0.086	0.406 (0.297 - 0.554)	*CTDSPL2*
12	rs4091651	1,88E-08	96880756	G/A	0.332/0.255	1.597 (1.356 - 1.880)	*CFAP54*
2	rs1549343	2,05E-08	171093785	G/A	0.283/0.185	1.625 (1.371 - 1.926)	*MYO3B*
6	rs241412	2,18E-08	32861616	T/G	0.422/0.323	1.552 (1.330 - 1.810)	*PSMB8; PSMB8-AS1; TAP1; PSMB9; LOC100294145; HLA-DMB*

(continued)

Table. List of the 26 AD-Associated SNPs That Pass the GWAS Significance Threshold.[a] *(continuation)*

CHR	SNP	*P* Value[b]	BP (GRCh37)	Minor/ major allele	MAF (case/ctrl)	OR (95%CI)[c]	Genes within 50kb of SNP
16	rs77286017	2,45E-08	70738119	A/G	0.043/0.090	0.435 (0.325 - 0.583)	*IL34; MTSS2; VAC14*
12	rs11170867	3,44E-08	54717410	C/T	0.168/0.112	1.845 (1.484 - 2.293)	*CBX5; SCAT2; HNRNPA1; NFE2; COPZ1; MIR148B; GPR84-AS1; GPR84; ZNF385A*
13	rs2057412	3,99E-08	49982570	C/T	0.079/0.147	0.538 (0.431 - 0.671)	*CAB39L; SETDB2; SETDB2-PHF11*
1	rs6426281	4,97E-08	246310557	G/A	0.071/0.137	0.501 (0.391 - 0.642)	*SMYD3*

Abbreviations: CHR, chromosome; SNP, single-nucleotide polymorphism; BP, base pair, Genome Reference Consortium Human Build 37; MAF, minor allele frequency in case/control.

[a]Gene annotations as per NCBI RefSeq track.

[b]*P* value: adjusted logistic regression *P* value.

[c]OR: adjusted odds ratio, minor allele as reference.

(DMEM, HyClone™; Cytiva) supplemented with 10% heat-inactivated fetal bovine serum (Gibco). Cells were cultured in a humidified incubator at 37°C with 5% CO_2 and passaged using trypsin (HyClone™; Cytiva) when they reached 90% confluency (up to 20 passages).

The *TJP2* gene promoter region from 69 119 282 to 69 121 282 bp (Chr9, GRCh38/hg38) of the top 2 most frequently occurring haplotypes in the population was cloned into promoter-less pGL4.10 plasmids with the firefly luciferase reporter gene (Promega). Cells were seeded at a density of 2.5 × 10^5 cells in 24-well plates and cultured for 24 hours followed by replacement with fresh cell culture medium before transfection. In the evaluation of the methylation inhibition effect, the medium was replaced with fresh medium containing 0 µM, 0.1 µM, 0.5 µM, or 1.0 µM of 5-aza-2'-deoxycytidine (DAC) prior to transfection with reporter constructs.

HEK293T cells were transiently transfected at 70%-80% confluency using Lipofectamine 2000 reagent (Invitrogen, Singapore) with 2 µg of reporter constructs according to the manufacturer's protocol. Four nanograms of control plasmid pGL4.74 with Renilla luciferase reporter gene was also co-

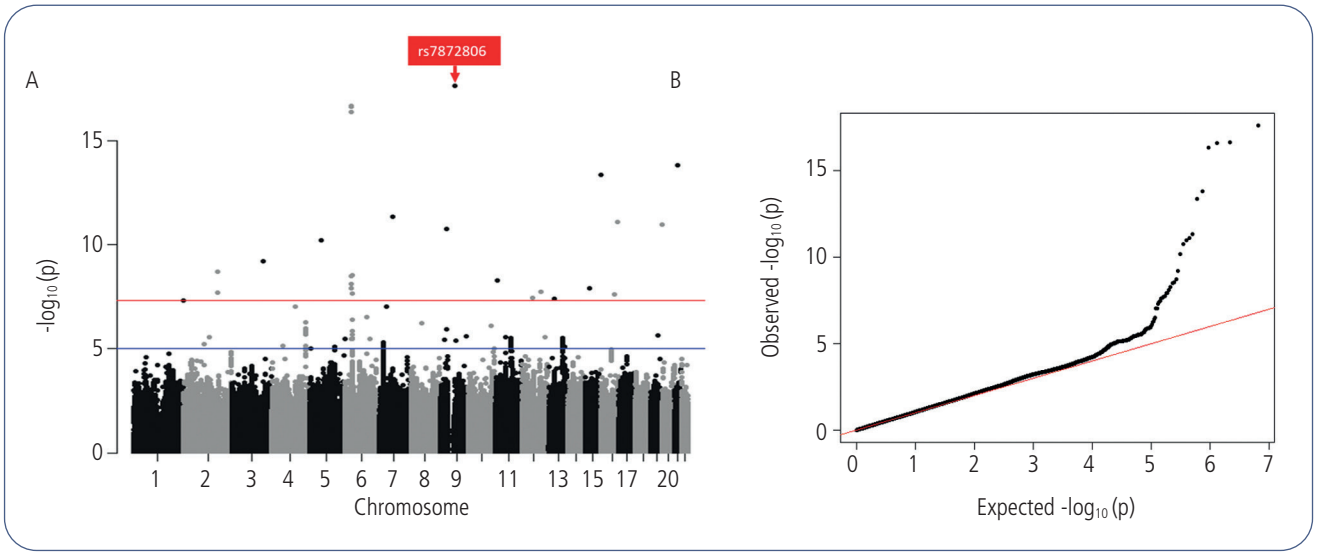

Figure 1. Genome-wide association study (GWAS) for 956 Singapore Chinese atopic dermatitis cases and 723 nonatopic controls. A, Manhattan plot showing the -log10 P values of 5 215 687 single-nucleotide polymorphisms (SNPs) in the cohort plotted against their respective positions on autosomes, after adjusting for age, sex, and PC1-10. SNPs with minor allele frequency <5% were excluded. The red line indicates the genome-wide significance threshold for this study ($P = 5 \times 10^{-8}$). The blue line shows the GWAS suggestive threshold ($P = 1 \times 10^{-5}$). $\lambda_{GC} = 1.048$. Twenty-six independent signals with GWAS $P < 5 \times 10^{-8}$ were obtained. Independent rs7872806 signal of interest labeled in red box. B, Quantile-quantile plot of *P* values observed plotted against expected. The red line indicates values predicted for a normal distribution. The probability plot illustrates that the GWAS data follow a normal distribution.

transfected to normalize for transfection efficiency. Cells were harvested 24 hours after transfection. Luciferase activity was measured using the Promega GloMax® Discover Microplate Reader and Dual Luciferase Reporter Assay Kit (Promega) according to the manufacturer's instructions. Firefly luciferase activity was normalized to that of Renilla to obtain the relative luciferase units (RLU). Transfection with empty vector pGL4.10 [luc] was also included as a control. All experiments were performed in triplicate.

Physiological Skin Parameters

Data on water barrier function (transepidermal water loss [TEWL]) were obtained using the Tewameter® TM300 device (Courage+Khazaka electronic GmbH) for 72 individuals from the Singaporean and Malaysian Chinese cohort at the antecubital fossa of both arms following 20 minutes of acclimatization according to the manufacturer's instructions. The average triplicate measurement was used for downstream analysis.

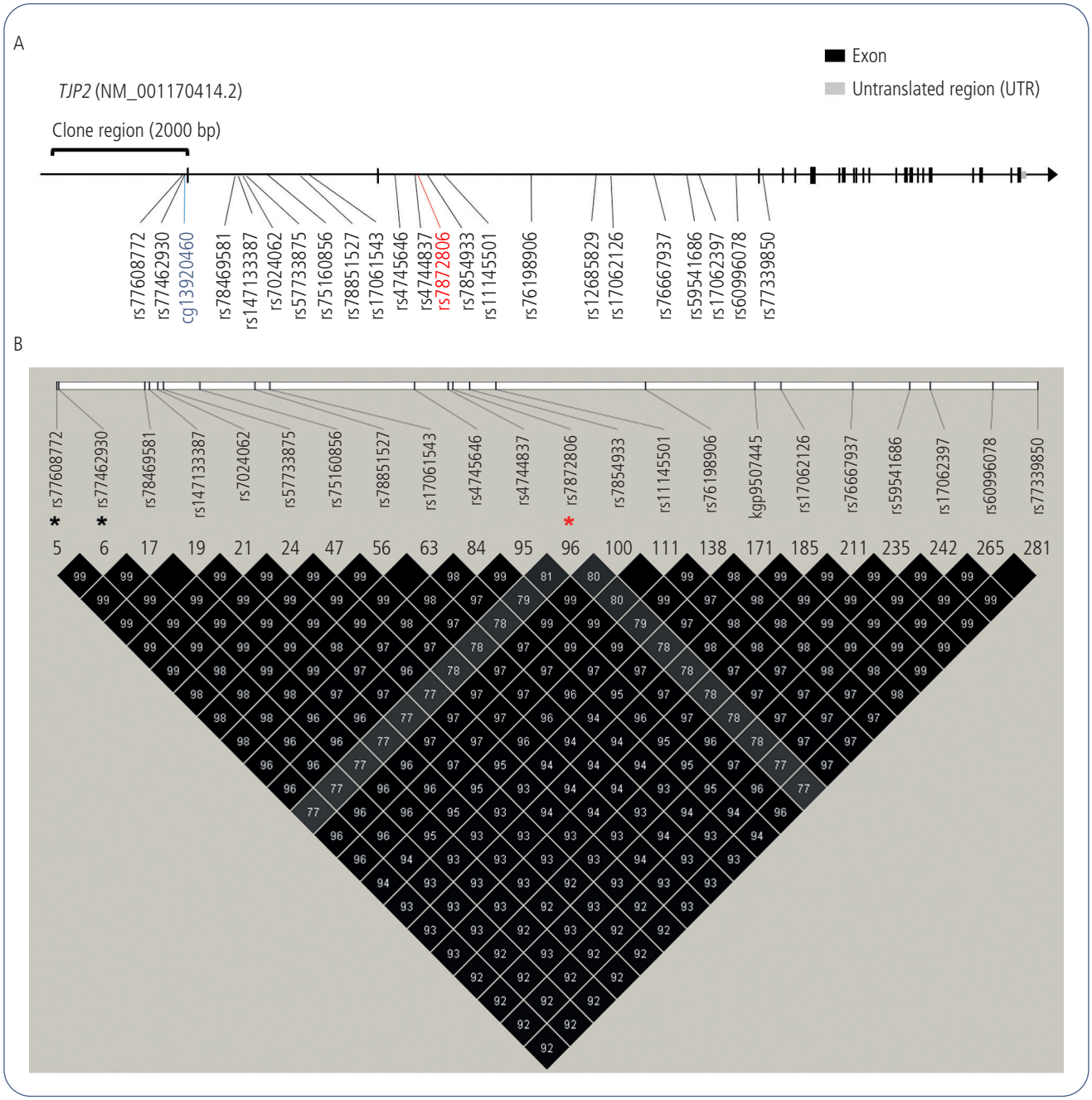

Figure 2. Schematic representation of single-nucleotide polymorphisms (SNPs) in the *tight junction protein 2* (*TJP2*) gene generated using Haploview® version 4.2. R^2 values are displayed as percentages. A, *TJP2* transcript variant NM_001170414.2 gene structure with the relative positions of 21 SNPs in high linkage disequilibrium (LD: $r^2 > 0.75$) with tag-SNP rs7872806 (red font) found in Singaporean and Malaysian Chinese individuals. CpG site cg13920460 is also indicated (blue font). B, LD pattern for all 21 SNPs in LD with tag-SNP rs7872806 (red asterisk) in the region of *TJP2* gene ±2 kb associated with atopic dermatitis in Singaporean and Malaysian Chinese individuals. The black asterisk indicates SNPs in the promoter region of the *TJP2* gene.

Results

Independent Genomic Signal at 9q21.11 (rs7872806) Associated With Atopic Dermatitis

A GWAS of 956 AD cases and 723 nonatopic controls with common SNP variants (ie, those with MAF ≥0.05) yielded a λ_{GC} of 1.048. The study identified 26 independent association signals at the stringent genome-wide significance threshold (GWAS $P<5 \times 10^{-8}$) (Figure 1A and B, Table), from which the rs7872806 minor allele "A" was found to have the strongest association with a reduced risk of AD (adjusted P value = 2.40×10^{-18}; OR, 0.38). Its minor allele frequency of 0.20 in the cohort we sampled is similar to that reported in East Asians from the 1000 Genomes project. LD analysis identified 21 other SNPs with strong pairwise correlations (LD: $r^2 > 0.75$) to SNP rs7872806. These SNPs were located in either intronic or promoter regions of *TJP2* transcript variant 5 (GenBank accession: NM_001170414.2) (Figure 2A and B, Table S2).

TJP2 SNP rs7872806 Is Associated With Higher TJP2 Expression in PBMCs but Not in the Epidermis

Given that the SNP rs7872806 lies in the deep intronic region of the *TJP2* gene (Figure 2A), we next examined whether it had any effect on *TJP2* gene expression. Using GTExPortal version 8, which contains *TJP2* expression data in various human tissues, we found SNP rs7872806 to significantly influence *TJP2* transcript expression only in whole blood (GTExPortal version 8 of 670 PBMCs, $P = 1.90 \times 10^{-7}$) but not in the skin epidermis, despite expression being much higher in the skin (GTExPortal version 8, Figure S1). To verify this, we analyzed *TJP2* transcript expression in PBMCs from 575 of the genotyped participants. Significantly higher *TJP2* transcripts (FPKM) were found with an increasing number of the minor allele "A" (Figure 3A, $P<0.01$ [*t* test]). This differential expression pattern in PBMCs was likewise reported in the eQTLGen Phase II database of 30 942 PBMCs ($P = 1.05 \times 10^{-119}$). Similarly, no disease-*TJP2* transcript expression association was observed in skin biopsies from 3 independent studies [26-28] (Figure S2). Covariates were not included in the analysis, as their incorporation did not influence the significance of our results.

Based on an additional literature search, we found that the *TJP2* protein had alternative functions in intracellular signaling. *TJP2* was found to be a chief regulator of several processes controlling cell proliferation rates, size, and number, through its association with nuclear protein activator protein 1 (AP-1), which regulates cyclin D1 (CD1) transcription [29,30]. We thus investigated the AP-1 and CD1 transcript levels in our PBMC samples and found an inverse relationship to *TJP2* expression concordant with our hypothesis ($P<.001$ [*t* test], Figure S3).

TJP2 SNP rs7872806 Is Associated With Reduced Methylation of CpG Site cg13920460

Since SNP rs7872806 is deep intronic and lies >37 kb from the start of the *TJP2* gene, it is unlikely to influence splicing or transcription factor binding. Nevertheless, previous studies have demonstrated a link between genotypic variation in noncoding regions and the regulation of DNA methylation and disease, most of which are cis-effects [31-33]. We thus investigated whether allele-specific methylation could account for the differential expression observed. We did so by analyzing the DNA methylation levels of CpG sites in the 10-kb region flanking the *TJP2* gene and checking for an association between methylation levels and the rs7872806 genotype.

After Bonferroni correction for multiple testing ($P<.05/67$ CpG sites) of the 6 CpG sites with significant associations with the rs7872806 genotype, only CpG site cg13920460 located 497bp upstream of the *TJP2* gene (Figure 3B, blue arrow, $P=2.12 \times 10^{-11}$) showed a significant negative correlation with *TJP2* expression in PBMCs (Pearson $P=1.09 \times 10^{-6}$, Pearson R=–0.313, Figure 3C). The minor allele "A" of rs7872806 was significantly associated with reduced methylation at CpG site cg13920460 ($P<.001$) and higher *TJP2* expression than the major allele (Figure 3C, 3D). The findings demonstrate robustness, even when a wider methylation window is applied and when covariate and cell heterogeneity controls are included (data not shown).

CpG Site cg13920460 In Vitro Methylation Effect on TJP2 Expression

We further sought to validate the effect of methylation on *TJP2* promoter activity in vitro by performing a luciferase assay. Three SNPs were identified to differ between the 2 most frequently occurring *TJP2* promoter haplotypes in the Chinese population residing in Singapore: haplotype 1 tagged to the rs7872806 "G" major risk allele (58.5% frequency) and haplotype 2 tagged to the rs7872806 "A" minor protective allele (24.8% frequency) (Figure 4). Plasmid constructs were generated for both haplotypes (region Chr 9: 69 119 282 – 69 121 282 bp, GRCh38/hg38) and cloned into promoterless pGL4.10 luciferase reporter gene plasmids. Basal RLU expression levels were very low in 24-hour posttransfected HEK293T cells, although exposure to DAC treatment inhibiting in vitro methylation led to a significant increase in the activity of haplotype 1 (G allele) promoter with a dose response, thus confirming the involvement of DNA methylation in regulating *TJP2* expression ($P<.05$ [*t* test], Figure 5A).

To determine whether the identified CpG site cg13920460 is consequential in regulating *TJP2* promoter activity, it was deliberately eliminated by replacing the cytosine base with a thymine, cloned into another pGL4.10 plasmid, and similarly transfected into HEK293T cells. The elimination of the single CpG site led to a significant increase in RLU expression levels of haplotype 1 (G allele) by about 25% to levels resembling that of haplotype 2 (A allele). In other words, CpG site cg13920460 plays a considerable role in regulating *TJP2* expression in mammalian cells through methylation ($P<.01$, Figure 5B and 5C).

SNP rs7872806 Is Associated With Reduced Transepidermal Water Loss in Skin

Given the predicted function of *TJP2* in influencing cell proliferation rates, we hypothesized that this contributes to the epidermal hyperplasia phenotype commonly observed in AD patients. Studies performed by Robles et al [34] and Pierce et al [35] also showed that overexpression of *CD1* led to the

presentation of epidermal hyperproliferation and hyperplasia in the skin of transgenic mice. Our hypothesized pathway of how SNP rs7872806 confers AD risk and begets the pathogenic phenotype is shown in Figure 6. Since studies have previously demonstrated the association between epidermal hyperplasia and significantly higher TEWL levels [36-38], we used TEWL measurements as a noninvasive approach to envisage the presence of skin barrier dysfunction.

Physiological skin TEWL levels of 72 participants were measured at the antecubital fossa, a region commonly implicated in the disease. In concordance with our data, individuals carrying the minor allele "A" at SNP rs7872806,

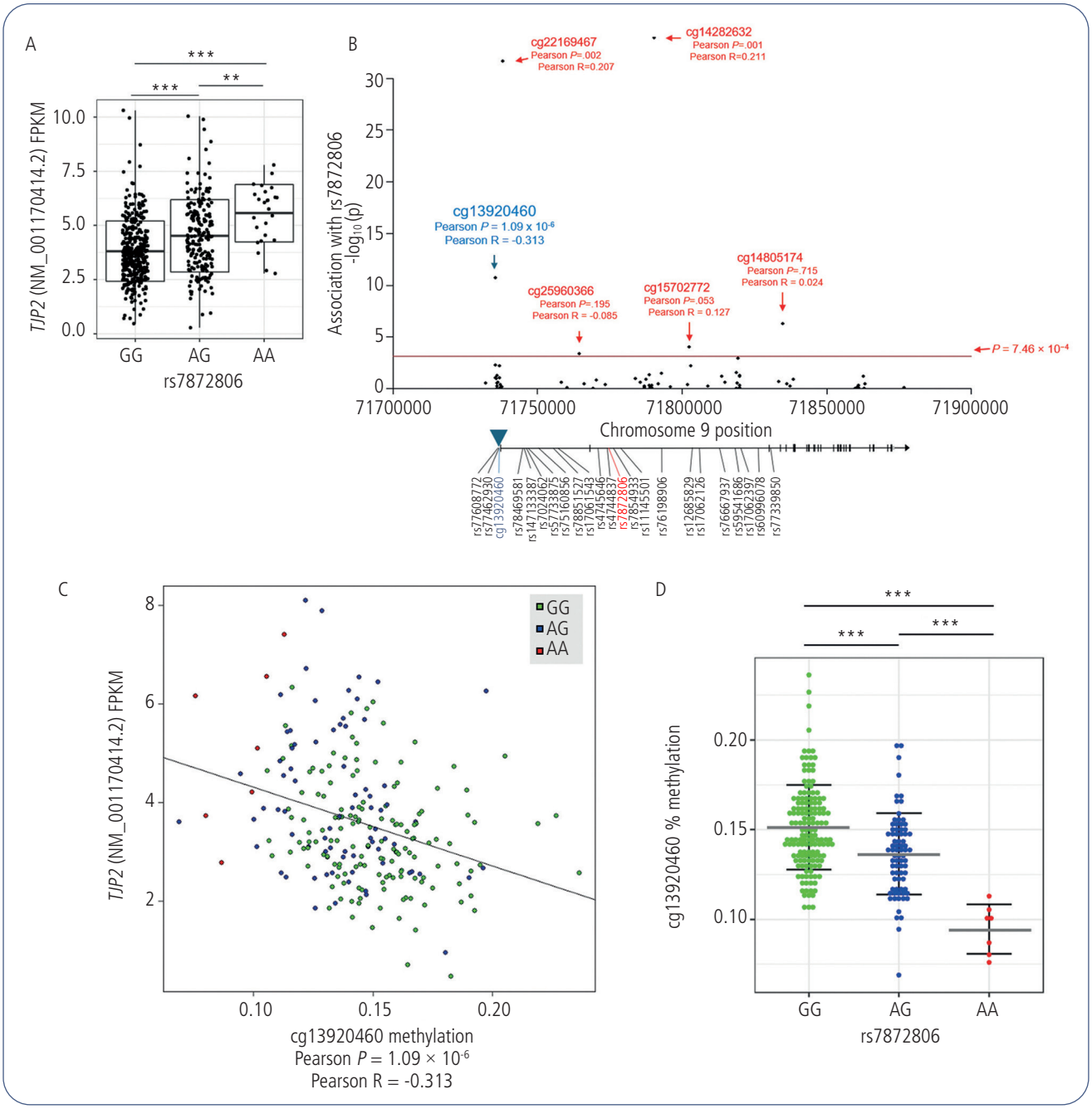

Figure 3. Effect of the TJP2 SNP rs7872806 genotype on gene expression. A, TJP2 transcript expression shown as quantile-normalized fragments per kilobase of transcript per million mapped reads (FPKM) in peripheral blood mononuclear cells (PBMCs) of 575 Singaporean and Malaysian Chinese grouped according to rs7872806 genotype. ***P*<.01 (*t* test). ****P*<.001 (*t* test). B, Association of SNP rs7872806 with 67 CpG sites within the 10-kb region flanking TJP2 gene measured in PBMCs of 233 Singaporean and Malaysian Chinese individuals. Blue arrow and font indicate CpG site of interest. C, Negative correlation between CpG site cg13920460 methylation levels and TJP2 transcript levels, which are shown as quantile-normalized FPKM. The negative correlation is significant at *P*<.05 (Pearson). D, Methylation levels at CpG site cg13920460 in PBMCs of 233 Singaporean and Malaysian Chinese individuals grouped according to the rs7872806 genotype. ****P*<.001 (*t* test).

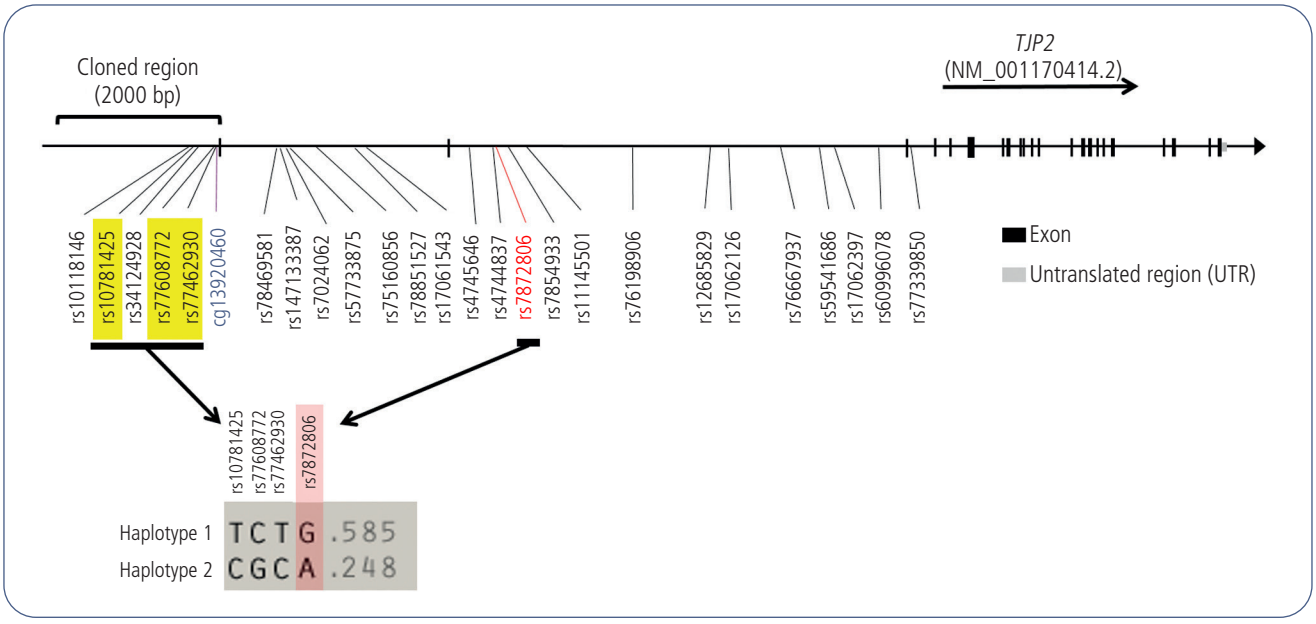

Figure 4. Gene structure of the TJP2 transcript variant NM_001170414.2 cloned region for the promoter-luciferase assay. Relative positions of common SNPs (>5% frequency) in the -2 kb region of the *TJP2* gene. The frequency for the top 2 most frequently occurring haplotypes in the Singaporean and Malaysian Chinese population is shown, generated from 3 SNPs in the promoter region (yellow highlighted) cloned for the promoter-luciferase assay. Haplotypes 1 and 2 tagged with the rs7872806 major G allele and minor A allele, respectively (red highlighted).

which was associated with higher *TJP2* expression, had significantly lower TEWL levels than those with the major "G" allele ($P<.001$, Figure 7).

Discussion

Previous GWAS have identified several AD-associated variants in populations of different ancestries. However, given the heterogeneity of the disease, several risk variants have yet to be identified and functionally characterized. The functional roles of deep intronic variants are also difficult to elucidate. In this study, we demonstrated the epigenetic control of a deep intronic polymorphism at 9q21.11 (SNP s7872806) in *TJP2* expression and AD disease.

The *TJP2* SNP rs7872806 was strongly associated with AD in our cohort of the Chinese population residing in Singapore, where its minor allele "A" conferred a protective effect against the disease by 2.64-fold. Given that the *TJP2* protein is expressed at high levels in the skin, its typically hypothesized AD-related function would have been structural. The tight junction proteins, also known as zonula occludens, are members of the family of membrane-associated guanylate kinase homologs that serve as a scaffold for assembly of other tight junction proteins, bridging the connection between claudins and the actin cytoskeleton in the cytoplasm [39].

Investigations into tissue-specific expression of *TJP2* in GTExPortal version 8, however, revealed an interesting association with the rs7872806 genotype only in whole blood but not in the skin (Figure S1). Similarly, in the eQTLGen Phase II database of 30 942 PBMCs, the same differential expression pattern was reported in whole blood but not in the skin. This was also replicated in the PBMCs extracted from the participants in our study, where *TJP2* transcript expression was higher in those carrying the minor allele "A" (Figure 3A). We thus performed an additional literature search into alternative functions of *TJP2* and found it to also be involved in intracellular signaling. The association between signaling molecules and AD is not a novel one, as demonstrated by Gamez et al [40], who established a connection between elevated levels of epithelial cell-derived cytokines to the early onset of AD. In a review by González-Mariscal et al [41], *TJP2* was described to be a chief regulator of several processes controlling cell proliferation rates, size, and number by associating with nuclear proteins. In a study conducted by Betanzos et al [42], overexpression of *TJP2* in Madin-Darby canine kidney (MDCK) cells led to a decrease in AP-1–controlled promoter activities in a dose-dependent manner. These sites, when bound by AP-1 transcription factors, can result in up-regulation of downstream proteins such as CD1. AP-1 binds 2 regulatory regions in the promoter of CD1 [43] and induces its transcription, thereby promoting cell cycle G1 progression and cell proliferation [44,45]. Given that patients with AD present with a significantly higher index of proliferative cells than healthy controls [46], we hypothesize that the effect of *TJP2* on the AD phenotype might be through AP-1 and CD-1. This was supported by the studies of Girolomoni and Pastore [47], where *AP-1* activation was found to be upregulated in AD keratinocytes, and of Kim et al [48], where *CD1* expression was higher in chronic psoriasis, whose phenotype is similar to that of AD, than in control skin. Mao et al [49] also identified that the drug cyproheptadine, which is commonly used to treat AD, had an inhibitory effect on *CD1* expression. In vitro studies

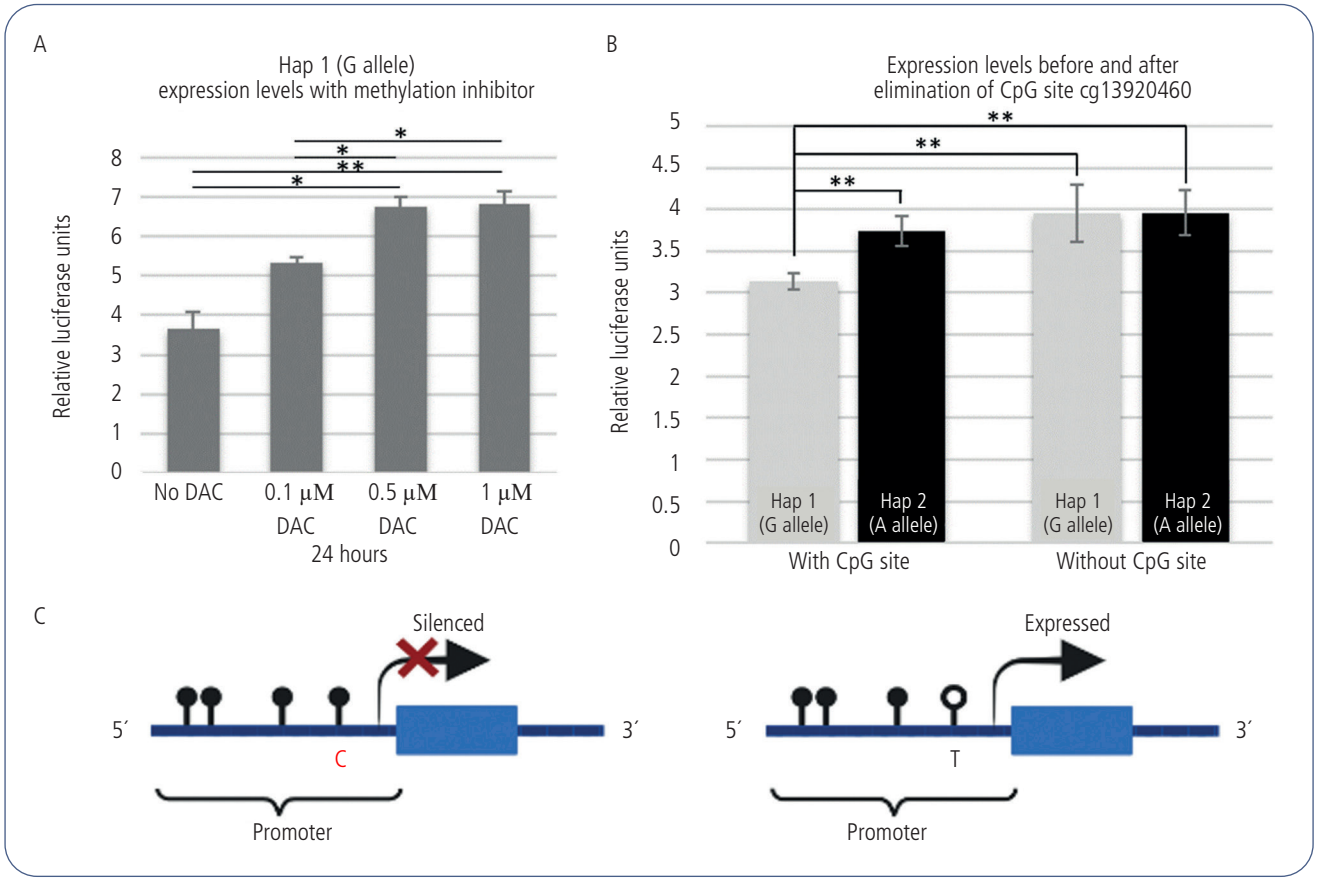

Figure 5. Influence of vitro methylation on TJP2 promoter activity. A, Increasing relative luciferase units of TJP2 haplotype 1 (G allele) promoter construct activity in the presence of 0 µM, 0.1 µM, 0.5 µM, or 1.0 µM of 5-aza-2'-deoxycytidine (DAC) assayed using HEK293T cells 24 hours post-transfection. Data represent 3 independent experiments with triplicate measurements normalized to pGL4.10 empty vector expression. *P<.05 (t test). **P<.01 (t test). B, Higher relative luciferase units of TJP2 haplotype 1 (G allele) promoter construct activity following elimination of CpG site cg13920460, to levels resembling that of haplotype 2 (A allele), assayed using HEK293T cells 24 hours post-transfection. Data represent 3 independent experiments with triplicate measurements normalized to pGL4.10 empty vector expression. **P<.01 (t test). C, Graphical illustration of the elimination of CpG site cg13920460 at the *TJP2* gene promoter. Change from a cytosine to a thymine base excludes methylation at that site and subsequently allows for the increased expression of the *TJP2* gene. Colored circles represent methylated sites, while open circles represent unmethylated sites. Cytosine and thymine are represented by "C" and "T", respectively.

investigating the knockdown of *TJP2* in MDCK cells have also found an increase in *CD1* expression [29]. From the PBMC samples of the participants in our study, we also observed a significant inversely proportional relationship between *TJP2* and *AP-1* or *CD1* expression (Figure S3).

Taking these findings together, we hypothesize that low levels of *TJP2* protein act to increase the activity of *AP-1* and, subsequently, the transcriptional activity of *AP-1*–regulated promoters, one of which is *CD1*. Therefore, the increase in *CD1* expression results in abnormally elevated cell proliferation rates and numbers, giving rise to epidermal hyperplasia, which is a characteristic phenotype in AD patients (Figure 6). The relationship between elevated CD1 levels and epidermal hyperproliferation was also demonstrated in a study by Robles et al [34], where *CD1* was overexpressed in the skin of transgenic mice. Given that several studies have demonstrated an association between the presence of epidermal hyperplasia and high TEWL levels in the skin [36-38], we measured TEWL levels in the skin of participants as a noninvasive approach to determining the existence of skin barrier dysfunction and epidermal hyperplasia. From the data collected, we were able to show that individuals carrying the major "G" allele of SNP rs7872806 had significantly higher antecubital fossa TEWL levels, with none of the individuals carrying the "A" allele presenting with TEWL levels above 23.19 $g \cdot m^{-2} \cdot h^{-1}$ (Figure 7), a cut off previously determined by Montero-Vilchez et al [50] for moderate and severe AD, with 73.5% sensitivity.

Understanding that deep intronic SNPs such as rs7872806 are unlikely to influence splicing [51], we investigated whether they had a cis-regulated allele-specific methylation effect, which has been demonstrated in other studies investigating complex diseases [31-33]. This led to the discovery of associations between the SNP rs7872806 genotype and differential methylation levels at the CpG site cg13920460 located in the promoter region of the *TJP2* gene (Figure 3B) and which was significantly inversely correlated with *TJP2* gene expression in PBMCs (Figure 3C). The methylation effect was further validated through in vitro methylation inhibition assays using DAC in HEK293T cells with dose response (Figure 5A). Elimination of the single CpG site

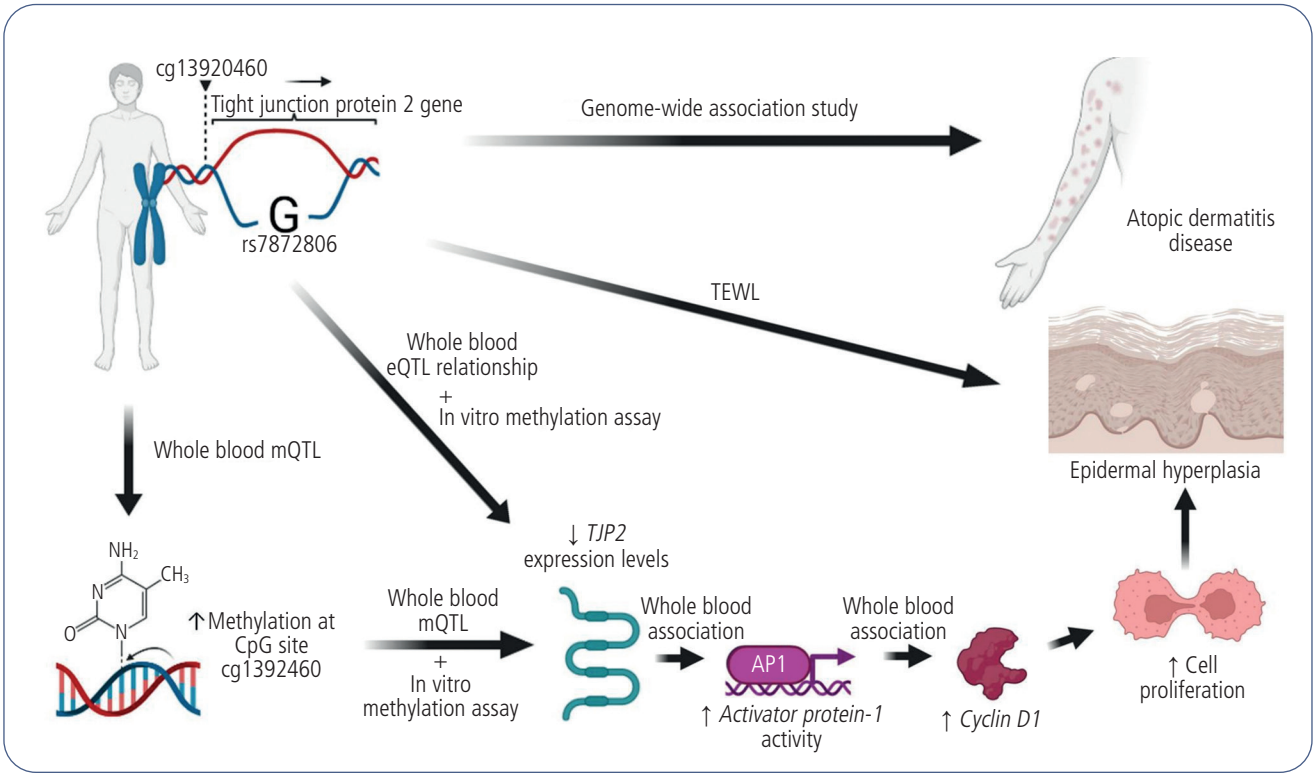

Figure 6. Summarized hypothesized pathway of the influence of rs7872806 on atopic dermatitis (AD). Major allele "G" of SNP rs7872806 found in the deep intronic region of the *tight junction protein 2* (*TJP2*) gene increased the risk of AD 3.25-fold through a genome-wide association study. The major "G" allele of SNP rs7872806 increases methylation levels at CpG site cg13920460, resulting in reduced *TJP2* expression levels and subsequently raising *activator protein-1* and *cyclin D1* activity and promoting increased cell proliferation and epidermal hyperplasia. mQTL indicates methylation quantitative trait loci; eQTL, expression quantitative trait loci; TEWL, transepidermal water loss.

cg13920460 was also sufficient to significantly raise the RLU value (Figure 5B and 5C), indicating a considerable role of this site in regulating *TJP2* expression. The complete hypothesized pathogenic pathway is shown in Figure 6.

To gain further insight into our genetic variant, we explored the FUMA GWAS platform (https://fuma.ctglab.nl/snp2gene) and noted a publication by Ter Hark et al [52] on LD variant rs7024062 of the SNP rs7872806. The authors reported a significant association between the major allele of rs7024062 and antipsychotic drug-induced weight gain (*P* value = 2.2×10^{-6}). In other case studies, such as that of Bujor et al [53], antipsychotic-treated patients who experienced weight gain also developed psoriatic lesions, suggesting that the major allele of rs7024062 may also be associated with psoriatic lesion formation. Given that epidermal hyperplasia is a hallmark of psoriatic skin lesions, the literature findings further strengthen our hypothesis that the major allele of the SNP rs7872806 contributes to the pathological pathway of AD through epidermal hyperplasia. Despite an extensive literature search, this was the only finding pertaining to the leading variant or its LD variants. The scarcity of research highlights the limited exploration of this specific genetic variant.

While the other SNPs in LD with rs7872806 exhibited patterns of *TJP2* transcript expression and allele-specific methylation that were identical to those of rs7872806 (data not shown) owing to their high LD ($r^2 > 0.75$), we hypothesize that rs7872806 is the SNP with the highest likelihood of being causal. This is because the GWAS association *P* value for rs7872806 was over 10^{14}-fold more significant in relation to the disease than its LD variants, strongly indicating its potential role as the causal site or the site with the most substantial effect size. However, we acknowledge that definitive confirmation of rs7872806 as the causal site would require further targeted in vitro perturbations, since our in vitro studies currently evaluate methylation effects as a haplotype.

Our study is subject to a series of limitations, one of which is the absence of replication. Following the approach advocated by Skol et al [54], which underscores the advantages of joint data analysis over replication-based methods owing to its higher statistical power, we opted to pool all available samples for our GWAS. To address the need for replication, we conducted an in silico search of various public GWAS databases (UK Biobank, Finngen, Biobank Japan Pheweb, MGL PheWeb). Unfortunately, we did not find any prior reports supporting the significance of rs7872806 in these datasets. For instance, in the Biobank Japan Pheweb GWAS, which included 4296 AD cases and 163 807 controls, the SNP rs7872806 yielded a *P* value of 6.3×10^{-1} with a minor allele effect size of –0.015 [55]. Similarly, the MGL PheWeb GWAS, encompassing 5018 AD/contact dermatitis cases and 35 760 controls, yielded a P value of 8.9×10^{-1} and a minor allele effect size of –0.0041 for SNP rs7872806, none of which reached GWAS significance. This outcome is not entirely unexpected, given the genetic heterogeneity across populations of different

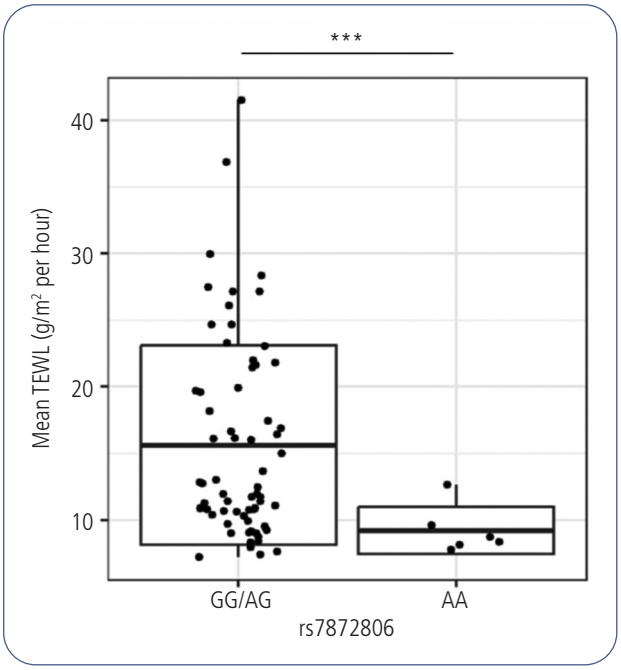

Figure 7. Presence of rs7872806 major "G" allele associated with a higher average transepidermal water loss (TEWL) level measured in the antecubital fossa of both arms of 72 Singaporean and Malaysian Chinese following 20 minutes of acclimatization. *P<.001 (t test).

ancestries. Replication in diverse populations is therefore challenging. Moreover, our proposed pathological pathway involves epigenetic mechanisms, which are strongly influenced by environmental factors that vary between populations. Future replication studies within the same population could offer further validation of our results.

Another aspect of our study that may raise concerns is the relatively small size of our control group (723 individuals vs 956 cases), as a result of our strict inclusion criteria for control participants. However, even if we were to expand the control group to include atopic individuals, increasing the control sample size to 2961, the result was an even more significant GWAS association P value of 5.40×10^{-39} for SNP rs7872806. This finding underscores that the smaller size of the original control group did not compromise the validity of our study. Nonetheless, we acknowledge the potential benefit of further increasing both the case and control sample sizes in future investigations to enhance the robustness of our findings.

In our in vitro expression studies, we made the unconventional choice of using HEK293T cells owing to the specific context and requirements of our study. The traditionally favored cell model for AD is keratinocytes, which are the primary cell type involved in AD-related skin barrier dysfunction and inflammation. However, since our investigation centers around the signaling function of the *TJP2* gene rather than its structural role, the observed effects need not be replicated in keratinocytes. Additionally, our early investigations of eQTL in keratinocytes from GTExPortal version 8 did not reveal significant differential *TJP2* expression associated with rs7872806 genetic variants in the skin. The use of PBMCs as a cell model was also ruled out owing to their heterogeneity, comprising a mix of various cell types. During our literature search, we came across a study conducted by Betanzos et al [42] that utilized MDCK cells as a model to investigate *TJP2* overexpression. This provided a precedent for exploring *TJP2* expression effects in epithelial-like kidney cells, such as HEK293T, which are of human origin. Moreover, HEK293T cells offer distinct advantages, including high transfection efficiency and proficient protein expression capabilities, attributes that make them ideal for gene expression experiments. In future research endeavors, further evaluation of *TJP2* expression in other cell models may offer a more comprehensive understanding of the pathogenesis of AD.

In addition to the results presented, we also conducted other analyses, although these failed to reveal significant associations. For instance, we explored potential correlations between disease phenotype and *TJP2* expression or cg13920460 methylation; however, our findings did not reach statistical significance. This may be due to the limited case sample size used for the RNAseq and methylation studies, coupled with the heterogenous and complex nature of AD, which can obscure direct associations. Nevertheless, the consistent findings from other evidence sources still provide confidence and reaffirm our overall conclusions. Expanding the cohort size in future works could enhance our ability to detect significance in these associations.

We also ventured to investigate whether there was a correlation between the frequency of the minor allele A of rs7872806 (retrieved from NCBI) and the reported prevalence of AD in various human populations [56,57], as documented in Supplementary Table S3, although our analysis yielded no correlation. Nevertheless, this lack of significant correlation aligns with our hypothesis that the pathological pathway of SNP rs7872806 is mediated through methylation, a process highly influenced by environmental factors that varies across populations.

In summary, this study identified and characterized a possible mechanism for the functional role of the polymorphism 9q21.11 in the AD phenotype. The SNP rs7872806 increases methylation at CpG site cg13920460, thereby influencing *TJP2* expression, which regulates downstream AP-1 and CD1 levels, resulting in increased cell proliferation and TEWL, which in turn points to epidermal hyperplasia. Our findings also provide insights into the epigenetic control of noncoding genetic polymorphisms and their ability to influence complex disease phenotypes. Such epigenetic mechanisms can thus be further investigated as potential targets for therapeutic intervention.

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Figure 6 was created with BioRender.com.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Cheok S, Yee F, Song Ma JY, Leow R, Ho MSL, Yew YW, et al. Prevalence and descriptive epidemiology of atopic dermatitis and its impact on quality of life in Singapore. Br J Dermatol. 2018;178(1):276-7.
2. Silverberg JI, Gelfand JM, Margolis DJ, Boguniewicz M, Fonacier L, Grayson MH, et al. Patient burden and quality of life in atopic dermatitis in US adults: A population-based cross-sectional study. Ann Allergy Asthma Immunol. 2018;121(3):340-7.
3. Lee BW, Detzel PR. Treatment of childhood atopic dermatitis and economic burden of illness in Asia Pacific countries. Ann Nutr Metab. 2015;66(1):18-24.
4. Elmose C, Thomsen SF. Twin studies of atopic dermatitis: Interpretations and applications in the filaggrin era. J Allergy. 2015;2015:1-7.
5. Matsuoka S, Nakagawa R, Nakayama H, Yamashita K, Kuroda Y. Prevalence of specific allergic diseases in school children as related to parental atopy. Pediatr Int. 1999;41(1):46-51.
6. Ober C, Yao TC. The genetics of asthma and allergic disease: A 21st century perspective. Immunol Rev. 2011;242(1):10-30.
7. Armario-Hita JC, Galán-Gutiérrez M, Dodero-Anillo JM, Carrascosa JM, Ruiz-Villaverde R. Updated review on treatment of atopic dermatitis. J Investig Allergol Clin Immunol. 2023;33(3):158-67.
8. Paternoster L, Standl M, Chen CM, Ramasamy A, Bøpnnelykke K, Duijts L, et al. Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. Nat Genet. 2012;44(2):187-92.
9. Ellinghaus D, Baurecht H, Esparza-Gordillo J, Rodríguez E, Matanovic A, Marenholz I, et al. High-density genotyping study identifies four new susceptibility loci for atopic dermatitis. Nat Genet. 2013;45(7):808-12.
10. Sun LD, Xiao FL, Li Y, Zhou WM, Tang HY, Tang XF, et al. Genome-wide association study identifies two new susceptibility loci for atopic dermatitis in the Chinese Han population. Nat Genet. 2011;43(7):690-4.
11. Schaarschmidt H, Ellinghaus D, Rodríguez E, Anja K, Baurecht H, Lipinski S, et al. A genome-wide association study reveals two new susceptibility loci for atopic dermatitis. J Allergy Clin Immunol. 2015;136(3):802-6.
12. Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, Sakashita M, et al. Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. Nat Genet. 2012;44(11):1222-6.
13. Tanaka N, Koido M, Suzuki A, Otomo N, Suetsugu H, Kochi Y, et al. Eight novel susceptibility loci and putative causal variants in atopic dermatitis. J Allergy Clin Immunol. 2021;148(5):1293-306.
14. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International study of asthma and allergies in childhood (ISAAC): Rationale and methods. Eur Respir J. 1995;8(3):483-91.
15. Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ, et al. The UK Working Party's diagnostic criteria for atopic dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. Br J Dermatol. 1994;131(3):383-96.
16. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol. 1980;60(92):44-7.
17. Lim JJ, Lim YYE, Ng JY, Malipeddi P, Ng YT, Teo WY, et al. An update on the prevalence, chronicity, and severity of atopic dermatitis and the associated epidemiological risk factors in the Singapore/Malaysia Chinese young adult population: A detailed description of the Singapore/Malaysia Cross-Sectional Genetics Epidemiology Study (SMCGES) cohort. World Allergy Organ J. 2022;15(12):100722.
18. Andiappan AK, Puan KJ, Lee B, Nardin A, Poidinger M, Connolly J, et al. Allergic airway diseases in a tropical urban environment are driven by dominant mono-specific sensitization against house dust mites. Allergy. 2014;69(4):501-9.
19. Roshyara NR, Kirsten H, Horn K, Ahnert P, Scholz M. Impact of pre-imputation SNP-filtering on genotype imputation results. BMC Genet. 2014;15(1):1-11.
20. Genomes Project C, Auton A, Abecasis GR, Altshuler DM, Durbin RM, Bentley DR, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.
21. Andiappan AK, Anantharaman R, Nilkanth PP, Wang DY, Chew FT. Evaluating the transferability of Hapmap SNPs to a Singapore Chinese population. BMC Genet. 2010;11:36.
22. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21(2):263-5.
23. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol. 2019;37(7):773-82.
24. Steen CB, Liu CL, Alizadeh AA, Newman AM. Profiling cell type abundance and expression in bulk tissues with CIBERSORTx. Methods Mol Biol. 2020;2117:135-57.

25. Rahmani E, Zaitlen N, Baran Y, Eng C, Hu D, Galanter J, et al. Sparse PCA corrects for cell type heterogeneity in epigenome-wide association studies. Nat Methods. 2016;13(5):443-5.
26. Plager DA, Leontovich AA, Henke SA, Davis MDP, Mcevoy MT, Sciallis GF, et al. Early cutaneous gene transcription changes in adult atopic dermatitis and potential clinical implications. Exp Dermatol. 2007;16(1):28-36.
27. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011;127(3):773-86.
28. Suárez-Fariñas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. J Allergy Clin Immunol. 2011;127(4):954-64.
29. González-Mariscal L, Miranda J, Raya-Sandino A, Domínguez-Calderón A, Cuellar-Perez F. ZO-2, a tight junction protein involved in gene expression, proliferation, apoptosis, and cell size regulation. Ann N Y Acad Sci. 2017;1397(1):35-53.
30. Huerta M, Muñoz R, Tapia R, Soto-Reyes E, Ramírez L, Recillas-Targa F, et al. Cyclin D1 is transcriptionally down-regulated by ZO-2 via an E Box and the transcription factor c-Myc. Mol Biol Cell. 2007;18(12):4826-36.
31. Meaburn EL, Schalkwyk LC, Mill J. Allele-specific methylation in the human genome: Implications for genetic studies of complex disease. Epigenetics. 2010;5(7):578-82.
32. Hutchinson JN, Raj T, Fagerness J, Stahl E, Viloria FT, Gimelbrant A, et al. Allele-specific methylation occurs at genetic variants associated with complex disease. PLoS One. 2014;9(6):e98464.
33. Johnson MD, Mueller M, Adamowicz-Brice M, Collins MJ, Gellert P, Maratou K, et al. Genetic analysis of the cardiac methylome at single nucleotide resolution in a model of human cardiovascular disease. PLoS Genet. 2014;10(12):e1004813.
34. Robles AI, Larcher F, Whalin RB, Murillas R, Richie E, Gimenez-Conti IB, et al. Expression of cyclin D1 in epithelial tissues of transgenic mice results in epidermal hyperproliferation and severe thymic hyperplasia. Proc Natl Acad Sci U S A. 1996;93(15):7634-8.
35. Pierce AM, Fisher SM, Conti CJ, Johnson DG. Deregulated expression of E2F1 induces hyperplasia and cooperates with ras in skin tumor development. Oncogene. 1998;16(10):1267-76.
36. Feingold KR, Mao-Qiang M, Proksch E, Menon GK, Brown BE, Elias PM. The lovastatin-treated rodent: A new model of barrier disruption and epidermal hyperplasia. J Invest Dermatol. 1991;96(2):201-9.
37. Imokawa G, Yada Y, Higuchi K, Okuda M, Ohashi Y, Kawamata A. Pseudo-acylceramide with linoleic acid produces selective recovery of diminished cutaneous barrier function in essential fatty acid-deficient rats and has an inhibitory effect on epidermal hyperplasia. J Clin Invest. 1994;94(1):89-96.
38. Fuziwara S, Suzuki A, Inoue K, Denda M. Dopamine D2-like receptor agonists accelerate barrier repair and inhibit the epidermal hyperplasia induced by barrier disruption. J Invest Dermatol. 2005;125(4):783-9.
39. Tsukita S, Furuse M. Pores in the wall: Claudins constitute tight junction strands containing aqueous pores. J Cell Biol. 2000;149(1):13-6.
40. Gamez C, Metcalfe J, Prescott SL, Palmer DJ. Circulating epithelial cell cytokines are associated with early-onset atopic dermatitis. J Investig Allergol Clin Immunol. 2022;32(1):48-50.
41. González-Mariscal L, Gallego-Gutiérrez H, González-González L, Hernández-Guzmán C. ZO-2 is a master regulator of gene expression, cell proliferation, cytoarchitecture, and cell size. Int J Mol Sci. 2019;20(17):4128.
42. Betanzos A, Huerta M, Lopez-Bayghen E, Azuara E, Amerena J, González-Mariscal L. The tight junction protein ZO-2 associates with Jun, Fos and C/EBP transcription factors in epithelial cells. Exp Cell Res. 2004;292(1):51-66.
43. Herber B, Truss M, Beato M, Müller R. Inducible regulatory elements in the human cyclin D1 promoter. Oncogene. 1994;9(4):1295-304.
44. Wisdom R, Johnson RS, Moore C. c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. EMBO J. 1999;18(1):188-97.
45. Albanese C, Johnson J, Watanabe G, Eklund N, Vu D, Arnold A, et al. Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions. J Biol Chem. 1995;270(40):23589-97.
46. Sapuntsova SG, Mel'nikova NP, Deigin VI, Kozulin EA, Timoshin SS. Proliferative processes in the epidermis of patients with atopic dermatitis treated with thymodepressin. Bull Exp Biol Med. 2002;133(5):488-90.
47. Girolomoni G, Pastore S. The role of keratinocytes in the pathogenesis of atopic dermatitis. J Am Acad Dermatol. 2001;45(1 Suppl):S25-8.
48. Kim SA, Ryu YW, Kwon JI, Choe MS, Jung JW, Cho JW. Differential expression of cyclin D1, Ki-67, pRb, and p53 in psoriatic skin lesions and normal skin. Mol Med Rep. 2018;17(1):735-42.
49. Mao X, Stewart AK, Hurren R, Gronda M, Lee K, Chow S, et al. A high throughput screen identified cyproheptadine that decreases D-type cyclins, arrests cells in the G1 phase, and induces apoptosis in multiple myeloma and leukemia cells. Blood. 2007;110(11):1507.
50. Montero-Vilchez T, Segura-Fernández-Nogueras MV, Pérez-Rodríguez I, Soler-Gongora M, Martinez-Lopez A, Fernández-González A, et al. Skin barrier function in psoriasis and atopic dermatitis: Transepidermal water loss and temperature as useful tools to assess disease severity. J Clin Med. 2021;10(2):359.
51. Woolfe A, Mullikin JC, Elnitski L. Genomic features defining exonic variants that modulate splicing. Genome Biol. 2010;11(2):R20.
52. Ter Hark SE, Jamain S, Schijven D, Lin BD, Bakker MK, Boland-Auge A, et al. A new genetic locus for antipsychotic-induced weight gain: A genome-wide study of first-episode psychosis patients using amisulpride (from the OPTiMiSE cohort). J Psychopharmacol. 2020;34(5):524-31.
53. Bujor CE, Vang T, Nielsen J, Schjerning O. Antipsychotic-associated psoriatic rash - a case report. BMC Psychiatry. 2017;17(1):242.
54. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet. 2006;38(2):209-13.

55. Sakaue S, Kanai M, Tanigawa Y, Karjalainen J, Kurki M, Koshiba S, et al. A cross-population atlas of genetic associations for 220 human phenotypes. Nat Genet. 2021;53(10):1415-24.
56. Hadi HA, Tarmizi AI, Khalid KA, Gajdács M, Aslam A, Jamshed S. The epidemiology and global burden of atopic dermatitis: A narrative review. Life. 2021;11(9):936.
57. Bylund S, Von Kobyletzki LB, Svalstedt M, Svensson Å. Prevalence and incidence of atopic dermatitis: A systematic review. Acta Derm Venereol. 2020;100(12):adv00160.

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Fook Tim Chew

Associate Professor, Department of Biological Sciences
Faculty of Science, National University of Singapore
Allergy and Molecular Immunology Laboratory
Lee Hiok Kwee Functional Genomics Laboratories
Block S2, Level 5, 14 Science Drive 4
Lower Kent Ridge Road, Singapore 117543
Tel: +65 65161685
E-mail: dbscft@nus.edu.sg