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Novel Role for Caspase Recruitment Domain Family Member 14 and its Genetic Variant rs11652075 in Skin Filaggrin Homeostasis

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Abstract

Background: Low epidermal filaggrin (FLG) is a risk factor for atopic dermatitis (AD) and allergic co-morbidity. *FLG* mutations do not fully explain the variation in epidermal *FLG* levels, highlighting that other genetic loci may also regulate *FLG* expression.

Objective: To identify genetic loci that regulate *FLG* expression and elucidate their functional and mechanistic consequences.

Methods: A genome-wide association study (GWAS) of quantified skin *FLG* expression in lesional and baseline non(never)-lesional skin of children with AD in the Mechanisms of Progression of Atopic Dermatitis to Asthma in Children (MPAACH) cohort was conducted. CRISPR-Cas9 approaches were used to create isogenic human keratinocytes differing only at the identified variant rs11652075, and *CARD14*-deficient keratinocytes for subsequent mechanistic studies.

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S.B.D.: designed and performed experiments, performed statistical analyses, wrote first draft of manuscript and other manuscript preparation; M.L.S.: assistance in experimental design and data analysis, editing of manuscript; H.H.: performed statistical analyses; J.M.B.: directed human subject studies, aided in statistical analysis and planning, editing of manuscript, secured funding for the study; J.W.K.: data management, performed statistical analyses; L.J.M.: designed and directed statistical analysis plans, editing of manuscript; G.K.K.H.: conceived overall experimental design and plan, writing and editing of manuscript, secured funding for the study.

Competing interests: The authors declare no conflicts of interest.

Results: The GWAS identified the *CARD14* rs11652075 variant to be associated with *FLG* expression in non(never)-lesional skin of children with AD. Rs11652075 is a *CARD14* eQTL in human skin and primary human keratinocytes. The T variant destroys a functional CpG site resulting in reduced CpG methylation at this site (but not neighboring sites) in TT and CT compared to CC primary human keratinocytes and MPAACH children's skin samples, and rs11652075 increases *CARD14* expression in an allele-specific fashion. Further, studies in CRISPR-generated CC and TT isogenic keratinocytes, as well as CARD14-haplosufficient and deficient keratinocytes, reveal that IL-17A regulates *FLG* expression via CARD14, and that the underlying mechanisms are rs11652075 genotype-dependent.

Conclusion: Our study identifies CARD14 as a novel regulator of *FLG* expression in the skin of children with AD. Further, *CARD14* regulates skin *FLG* homeostasis in a rs11652075-dependent fashion.

Clinical Implication: Our results highlight the CARD14 signaling pathway as a promising new target for therapeutic intervention in AD.

Capsule Summary:

Through a GWAS and subsequent functional analyses, the *CARD14* variant rs11652075 was found to regulate *FLG* in non-lesional skin of children with AD, thus identifying a candidate pathway for novel therapeutics.

Keywords

Atopic Dermatitis; Filaggrin; CARD14; GWAS; Skin; Gene; Skin barrier

Introduction:

Atopic dermatitis (AD), or eczema, is a chronic, relapsing inflammatory skin disease affecting 15-30% of children globally and often precedes development of allergic asthma and other atopic diseases in the "atopic march" (1-3). A hallmark characteristic of AD is a dysfunctional skin barrier, which allows for epicutaneous penetration of environmental allergens and subsequent allergic sensitization (2). Loss-of-function mutations in the gene encoding filaggrin (*FLG*), a cornified envelope protein critical for epidermal barrier formation and function, are the most significant genetic risk factors for AD (4). However, low *FLG* expression levels are common in AD even in the absence of such mutations (4).

We recently found that low non(never)-lesional, but not lesional, skin *FLG* expression is associated with development of co-sensitization and moderate-severe AD (5), both key risk factors for future development of allergic AD co-morbidities (6). The importance of non-lesional skin *FLG* expression level in determining AD outcomes was similarly reported in another recent study (7). As such, there is a critical need to delineate factors that regulate *FLG* expression in non-lesional skin of patients with AD (2).

Herein we utilized the Mechanisms of Progression of Atopic Dermatitis to Asthma in Children (MPAACH) early life cohort of children with AD (5) to perform a GWAS to identify genetic variants associated with epidermal *FLG* levels and identified the common

rs11652075 missense variant of the Caspase Activation and Recruitment Domain Family Member 14 (*CARD14*) gene as a novel genetic locus associated with reduced epidermal *FLG* expression in non-lesional skin. We found the variant destroys a CpG site and induces allelic *CARD14* expression in keratinocytes, suggesting the variant is a *CARD14* expression quantitative trait locus (eQTL). Finally, we show IL-17A, which activates CARD14 signaling in keratinocytes (8), suppresses *FLG* through CARD14 in a rs11652075 genotype-dependent manner. Taken together, our work identifies the *CARD14* gene and its variant rs11652075 as regulators of *FLG* mRNA levels within skin.

Methods

Study Design and Approval.

The GWAS utilized the MPAACH cohort, whose recruitment details, exclusion and inclusion criteria have been detailed previously (5) and are summarized in the Online Methods. The MPAACH study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board under protocol number 2016-5842, and informed consent was provided by all subjects.

Genome Wide Association Study.

To test for the association between *FLG* levels and single nucleotide polymorphisms (SNPs), linear regression models of log-transformed (to address normality) non-lesional *FLG* were performed in PLINK (Version 1.07). Age, sex and race were included as co-variates. For the lesional analysis, non-lesional *FLG* was an additional covariate to account for baseline differences. Multiple testing correction and several quality control metrics were employed during the analysis. Details of *FLG* measurement and statistical methodology are available in the Online Repository.

Other Methods:

Please see the Online Methods and Table E1 for a thorough description of all methods and materials.

Results:

The CARD14 *rs11652075 variant is associated with reduced non-lesional* FLG *levels in the epidermis of children with AD*

The importance of non-lesional skin *FLG* expression level in determining AD outcomes has been reported by our group and others (5,7). To elucidate genetic variants that are associated with skin *FLG* expression, we performed a GWAS on MPAACH participants using non-lesional or lesional *FLG* mRNA levels as a continuous outcome variable. Skin *FLG* mRNA levels were quantified from mRNA isolated from skin tape strips taken from lesional (site of active or historical lesion) and non(never)-lesional (no current lesion *and* no history of a lesion at this site *and* >10 cm from a lesional site) sites from each MPAACH participant at the enrollment visit as previously described (5). The first 240 participants enrolled in MPAACH who passed genotyping QC and had data available for non-lesional skin *FLG* expression level were included in this study. Previous studies have validated

the reproducibility and reliability of the *FLG* expression data (5), and that the skin tape strips sample stratum corneum keratinocytes (9). The characteristics of the genotyped cohort compared to the non-genotyped cohort and the overall cohort are detailed in Table E2. There was a small but significant difference in race.

Analyses of non-lesional *FLG* levels identified 2 SNPs, rs56344002 ($p=3.8x10^{-7}$) and rs11652075 ($p=4.2x10^{-7}$), that surpassed the multiple-testing correction significance threshold of $4.2x10^{-7}$ based on an LD-adjusted Bonferroni correction in order to account for correlation between variables and more appropriately control type I error than traditional Bonferroni correction, which assumes independence (10) (Figure 1A, Table E3). Rs56344002 is an intron variant located in *NEUROTRIMIN (NTM)*, and rs11652075 is a missense variant in *CARD14*. Imputation of SNPs in the *CARD14* and *NTM* genes reveals that there are several SNPs in moderate to strong LD with rs11652075 and rs56344002 that exhibit p-values $<10^{-4}$, further supporting the significance of these two variants (Figure E1) in non-lesional skin. Genetic association of lesional *FLG* levels identified no SNPs that surpassed the multiple testing threshold (Figure 1A; Table E4). Race-stratified analyses support similar effects in the black and non-black populations (Tables E3 and E4).

CARD14 *is highly expressed in the skin, where the rs11652075 T allele is a candidate tissue-specific* CARD14 *eQTL*

Rs11652075 is a C>T transition variant within exon 20 of the *CARD14* gene (Figure 1B). CARD14 is a scaffolding protein that, upon activation, nucleates the assembly of B-cell lymphoma/leukemia 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) into the CARD14-BCL10-MALT1 (CBM) complex which induces nuclear factor kappa B (NFkB) and mitogen activated protein kinase (MAPK) signaling (11-14). Notably, NFkB and MAPK signaling have been previously implicated in *FLG* regulation (15-23), but this has not been examined in the context of CARD14 signaling. *CARD14* variants, including rs11652075, are also associated with psoriasis, a distinct inflammatory skin disease that shares several characteristics with early-onset pediatric AD (11,24,25). Thus, we prioritized the rs11652075 variant for biologic investigation.

Publicly available Genotype-Tissue Expression (GTEx) data reveal that *CARD14* expression is highest in epithelial tissues including sun-exposed and non-sun-exposed skin and esophageal mucosa (>15 transcripts per million (TPM)), whereas its expression is low (<0.6 TPM) in nearby tissues including the esophageal muscularis, fibroblasts, and subcutaneous adipose (Figure 1C), consistent with previous data (12). Furthermore, the normalized effect size (NES) of the rs11652075 T allele demonstrates that the T allele is associated with higher *CARD14* expression in non-sun-exposed skin and the esophageal mucosa (Figure 1D). Conversely, the T allele is associated with reduced *CARD14* expression in several tissues, including the esophageal muscularis, fibroblasts, and subcutaneous adipose. These findings indicate that *CARD14* is highly expressed in the skin, and rs11652075 is a candidate tissue-specific *CARD14* eQTL.

The rs11652075 variant destroys a cytosine-phosphate-guanine (CpG) site within the CARD14 *gene*

To determine why the rs11652075 T allele is associated with increased skin *CARD14* expression (Figure 1D), we examined the variant's genomic context and found that the cytosine affected by rs11652075 is within a putative CpG site (Figure 2A). CpG cytosines can be enzymatically methylated to epigenetically alter chromatin conformation and gene transcription (26). Given that CpG polymorphisms are associated with epigenetic and transcriptional changes (27-29), we next examined methylation of the rs11652075 CpG site in primary keratinocytes from individuals of each genotype to determine if it is a functional CpG that is destroyed by the C>T transition. Primary keratinocyte donor characteristics are detailed in Table E5. Pyrosequencing of bisulfite-converted DNA revealed that the rs11652075 CpG is 86.5% methylated in homozygous C allele (CC) keratinocytes, but that this methylation is decreased to 39.2% and 8.5% in heterozygous (CT) and homozygous T allele (TT) keratinocytes, respectively (Figure 2B). Importantly, pyrosequencing of bisulfite-converted DNA isolated from skin tape strips taken from MPAACH children exhibited similar methylation trends, with CC subjects exhibiting 94.4% methylation at the rs11652075 CpG, but only 53.9% and 9.9% methylation in CT and TT subjects, respectively (Figure 2C). Four neighboring CpGs downstream of rs11652075 exhibited only minor methylation changes in primary keratinocytes and no detectable changes in human skin, indicating that the effects of rs11652075 on methylation are specific to the rs11652075 CpG (Figure E2A and Figure E2B). These data demonstrate that the rs11652075 CpG is functional but is destroyed by the C>T transition. This finding suggests epigenetic mechanisms may contribute to the differential *CARD14* expression observed in the GTEx data.

The rs11652075 T allele is expressed more efficiently than the C allele in keratinocytes

Since the rs11652075 T allele is associated with both increased *CARD14* expression in skin (Figure 1D) and altered *CARD14* methylation in primary keratinocytes and skin (Figure 2B and Figure 2C), we hypothesized that the transcriptional efficiency of *CARD14* is greater from the T allele compared to the C allele. Since the variant is exonic we utilized allele-specific qPCR (Figure 2D) to calculate the ratio of transcripts expressed from the two rs11652075 alleles (a T:C ratio) in primary keratinocytes derived from three heterozygous (CT) individuals (Table E5). Expression was consistently higher from the T allele with the T:C ratio from three separate donors (894, 904, and 929) being 1.5, 1.34, and 1.49, respectively (Figure 2E). These results, which corroborate the GTEx data (Figure 1D), suggest that the rs11652075 T allele is expressed more efficiently than the C allele in keratinocytes and may thus be a keratinocyte *CARD14* eQTL.

CARD14 mediates IL-17A-induced FLG *suppression in a rs11652075 genotype-dependent fashion*.

Since our GWAS identified the rs11652075 *CARD14* variant to be associated with reduced non-lesional *FLG*, we investigated whether CARD14 signaling directly mediates *FLG* suppression. The cytokine IL-17A triggers CARD14 signaling in keratinocytes via its receptor IL-17R (8), which is constitutively expressed on keratinocytes (30). Interestingly,

IL-17A is known to reduce keratinocyte *FLG* expression (15,16,31), and altered type 17 responses are implicated in AD pathogenesis—especially pediatric AD, which is the focus of the present study (25,32-37). For these studies we utilized HaCaT human keratinocytes, which are homozygous wild-type (CC) at rs11652075 (*CARD14*$^{C/C}$, Figure E3), cultured in high-calcium medium (1.9 mM) to promote a more differentiated state. We treated HaCaT human keratinocytes with IL-17A for 24 hours with and without mepazine, a compound that disrupts CARD14 signaling by inhibiting MALT1 enzymatic activity (38,39). IL-17A treatment reduced *FLG* by 20.0%; however, mepazine co-treatment failed to rescue *FLG* expression (Figure 3A), suggesting that IL-17A-induced *FLG* suppression is not mediated by CARD14 signaling through MALT1 enzymatic activity in *CARD14*$^{C/C}$ HaCaT keratinocytes. The CARD14 target genes *CCL20* and *CXCL8* (encoding IL-8) (13,40) were both induced by IL-17A, but *CXCL8* induction was prevented by mepazine co-treatment and a similar, but not significant, trend was observed for *CCL20* in the *CARD14*$^{C/C}$ HaCaT keratinocytes (Figure 3A). However, as these trends were observed in *CARD14*$^{C/C}$ HaCaT keratinocytes that lacked the rs11652075 T allele, we were prompted to consider if the mechanisms underlying IL-17A-induced *FLG* suppression were rs11652075 genotype-dependent.

To address this possibility, we stimulated primary human keratinocytes isolated from human donors of each rs11652075 genotype (Figure E3) with IL-17A ± mepazine and measured *FLG*, *CCL20*, and *CXCL8* expression. The CC keratinocytes exhibited a trend similar to the *CARD14*$^{C/C}$ HaCaT keratinocytes: IL-17A reduced *FLG* by 60.4% and was unaffected by mepazine co-treatment. In CT and TT keratinocytes, IL-17A also suppressed *FLG* by 45.1% and 50.3% respectively; however, their response to mepazine co-treatment was notably different than CC keratinocytes: mepazine co-treatment not just rescued *FLG*, but actually *induced FLG* expression in CT and TT keratinocytes by 2.61- and 1.55-fold, respectively (Figure 3B). Furthermore, IL-17A-dependent *CCL20* and *CXCL8* induction in CT and TT keratinocytes was more effectively blunted by mepazine; this was most evident in the TT keratinocytes. These data support a rs11652075 genotype-dependent mechanism regulating *FLG* downstream of IL-17A.

Since primary keratinocytes are derived from heterogeneic individuals, we cannot discount that variants distinct from rs11652075 contribute to the differential IL-17A response. We therefore used CRISPR-Cas9 to knock-in the rs11652075 T allele into the CC HaCaT keratinocytes, thus generating homozygous T allele (*CARD14*$^{T/T}$) HaCaT cells that are isogenic to *CARD14*$^{C/C}$ HaCaT cells (Figure E3). IL-17A treatment of *CARD14*$^{T/T}$ HaCaT keratinocytes resulted in a 33.9% reduction in *FLG*. However, similar to CT and TT primary keratinocytes but in contrast to *CARD14*$^{C/C}$ HaCaT cells and CC primary keratinocytes, mepazine co-treatment rescued *FLG* to above baseline, inducing its expression 1.21-fold (Figure 3C). Taken together, these results reveal that CARD14 signaling mediates IL-17A-induced *FLG* suppression in a rs11652075 genotype-dependent manner.

CARD14 *knockout abrogates* FLG *suppression in HaCaT keratinocytes*

To confirm that CARD14-mediated signaling is specifically involved in *FLG* regulation we used CRISPR-Cas9 to sequentially knockout both *CARD14* alleles in *CARD14*$^{C/C}$

(also designated as *CARD14*$^{+/+}$) HaCaT keratinocytes, thereby creating both *CARD14* haploinsufficient (*CARD14*$^{+/-}$) and deficient (*CARD14*$^{-/-}$) HaCaT lines (Figure E4). Since endogenous CARD14 protein is undetectable even in *CARD14*$^{+/+}$ HaCaT keratinocyte lysates, we confirmed knockdown efficacy by incorporating the CRISPR-Cas9-induced edits into CARD14-FLAG expression plasmids (Figure E5A), which failed to express CARD14 protein when transfected into HaCaT keratinocytes (Figure E5B). We observed a stepwise increase in baseline *FLG* expression with *CARD14* allele loss, with haploinsufficient and deficient HaCaT strains exhibiting 2.24- and 3.81-fold higher *FLG* relative to wild-type. Whereas IL-17A reduced *FLG* levels by 24.3% in *CARD14*$^{+/+}$ HaCaT keratinocytes, this effect was blunted to 19.4% in *CARD14*$^{+/-}$ HaCaT keratinocytes, and no significant reduction was detected in *CARD14*$^{-/-}$ HaCaT keratinocytes (Figure 4A). To evaluate the effects of CARD14 signaling on *FLG* expression *independent* of a cell-surface receptor (i.e. IL-17R), we treated *CARD14*$^{+/+}$ HaCaT keratinocytes with phorbol 12-myristate 13-acetate (PMA) and ionomycin with and without mepazine pre-treatment (40). PMA/ionomycin (PI) alone suppressed *FLG* by 67.0%, but mepazine rescued expression by 50.0% (Figure 4B). However, as observed with IL-17A, the use of *CARD14*$^{+/-}$ HaCaT keratinocytes blunted PI-induced *FLG* suppression to an only 38.7% reduction, and no significant change was detected in *CARD14*$^{-/-}$ HaCaT cells (Figure 4C). Taken together, these data confirm that CARD14 has a functional role in regulating the *FLG* gene in keratinocytes.

Discussion:

Herein, we conducted the first GWAS of skin *FLG* expression as an eQTL and identified *CARD14* as an important regulator of *FLG* expression in non-lesional skin. No variants were significantly associated with *FLG* levels in lesional skin, possibly due to the effects of local inflammation on *FLG* expression overpowering genetic effects (41). Further, we demonstrate that the *CARD14* rs11652075 variant is functional: the variant destroys a CpG and disrupts its normally high level of methylation, and *CARD14* is transcribed more efficiently from the risk (T) allele than reference (C) allele. Finally, we show that CARD14 signaling regulates *FLG* downstream of IL-17A, and that the underlying mechanisms are rs11652075-dependent. Together, these experiments suggest a model in which the rs11652075 T allele increases CARD14 levels and activity resulting in reduced *FLG* expression, contributing to the skin barrier defect observed in AD (Figure 5).

Our study is the first unbiased genetic association study to link *CARD14* and rs11652075 to skin barrier function and AD. This is likely due to our analytic approach, which aimed to identify variants associated with a specific quantitative AD risk factor (2) (*FLG* levels) rather than with AD presence versus absence (42). Only one previous study has examined *CARD14* in the AD context, which associated two *CARD14* loss-of-function variants with severe AD (43). However, the effects of these variants on *FLG* expression were not evaluated.

Our data clearly demonstrate that rs11652075 affects *CARD14* methylation and expression. However, we cannot exclude other potential mechanisms that contribute to the cumulative impact of rs11652075 on AD. In addition to transcriptional changes, rs11652075 is also a

missense variant (R820W) within the full-length CARD14 protein (24,44) and is thus likely to induce protein-level alterations. This is the focus of ongoing experiments.

Our study is the first to show that CARD14 signaling directly regulates *FLG* expression. Upon CARD14 activation and CBM assembly, MALT1 activates NFkB and major MAPK pathways (JNK, ERK and p38), and proteolytically inactivates enzymes that attenuate these pathways, such as A20 (11-14). Low A20 levels have been detected in the skin of human AD patients, and epidermal A20 deletion induces NFkB/MAPK activity and exacerbates experimental AD in mice (45). NFkB signaling is a known contributor to AD pathogenesis (46-48) and can suppress *FLG* (21). While JNK signaling induces *FLG* (18,19,22), conflicting reports exist regarding the impact of ERK and p38 on *FLG* expression (16,20,23). Notably, ERK and p38 suppress *FLG* in response to IL-17A (16), making them the likely pathways mediating *FLG* suppression downstream of CARD14. Furthermore, differential activation of these pathways may underly the rs11652075-dependent effects of CARD14 signaling on *FLG* regulation. Although *FLG* is expressed in monolayer, future experiments addressing these underlying mechanisms will utilize three-dimensional models (e.g., air-liquid interface and epidermal organoid models) that more optimally recapitulate *FLG* expression than monolayer culture, which is a limitation of the present study.

There is a growing appreciation for the role of IL-17A in AD (32-37). Elevated IL-17A levels have been observed in both the lesional and non-lesional skin of pediatric AD patients (25,37,49,50), and is thought to contribute to AD pathogenesis by promoting the differentiation of pro-allergic T-helper 2 cells (34) and by suppressing *FLG* in keratinocytes (15,16,31). While it is known that IL-17A stimulates CARD14 signaling (8), our study shows that CARD14 is necessary for IL-17A-induced *FLG* suppression. Furthermore, the effects of IL-17A on *FLG* appear to be rs11652075-dependent, with the T allele being associated with both reduced *FLG* levels and improved *FLG* rescue upon CBM inhibition. Our findings thus clearly demonstrate that CARD14 signaling is one mechanism by which IL-17A modulates keratinocyte *FLG* expression and contributes to AD pathogenesis.

IL-17A and the IL-23/IL-17A axis are also critical in the development of psoriasis and pityriasis rubra pilaris (PRP), two inflammatory skin diseases which, notably, are associated with *CARD14* mutations (24,51-54). Despite being distinct diseases, early-onset pediatric AD and psoriasis share similar inflammatory environments (25). An unsupervised clustering analysis revealed that the molecular profile of pediatric non-lesional AD is more similar to that of adult psoriatic skin than adult AD skin, including elevated IL-17A, CCL20 and IL-8 (37). Multiple reports have noted PRP patients with gain-of-function *CARD14* mutations who exhibit several AD-like features, including eczematous lesions, elevated total and allergen-specific IgE, and near absence of epidermal FLG (55,56). Aberrant CARD14 signaling may thus be the nexus between the pathogeneses of these distinct inflammatory skin conditions. Interestingly, the C allele of the rs11652075 variant has been linked to both psoriasis and PRP by several groups (54,57-67), though the underlying mechanisms are unclear. Further research is warranted to define the role of *CARD14* in the pathogenesis of AD versus that of other inflammatory skin diseases, and how its role is influenced or conditioned by the presence of the rs11652075 C versus T allele.

Our findings highlight a novel role of *CARD14* and the rs11652075 variant in the regulation of *FLG* in keratinocytes. Low epidermal *FLG* disrupts the skin barrier and is a significant risk factor for AD development (2,4,5). Since the T allele of rs11652075 is common (a 47.68% global minor allele frequency (68)), rs11652075 may contribute to reduced *FLG* levels and compromised barrier function in many individuals, underscoring the high impact of our studies. Our findings thus reveal the potential for targeting CARD14 signaling in the prevention or treatment of AD, particularly in CT and TT individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AD	Atopic Dermatitis
BCL10	B-cell lymphoma/leukemia 10
CARD14	Caspase recruitment domain family member 14
Cas9	CRISPR associated protein 9
CBM	CARD14-BCL10-MALT1
CCL20	C-C motif chemokine ligand 20
CpG	Cytosine-phosphate-guanine
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats
CXCL8	C-X-C motif chemokine ligand 8
eQTL	Expression quantitative trail locus
ERK	Extracellular signal-regulated kinase
FLG	Filaggrin
GWAS	Genome-wide association study
GTEx	Genotype-Tissue Expression
IL	Interleukin
JNK	c-Jun N-terminal kinase

LD	Linkage disequilibrium
MALT	Mucosa-associated lymphoid tissue lymphoma translocation protein 1
MAPK	Mitogen activated protein kinase
MPAACH	Mechanisms of Progression of Atopic Dermatitis to Asthma in Children
NES	Normalized effect size
NFkB	Nuclear factor kappa B
PI	PMA/ionomycin
PMA	Phorbol 12-myristate 13-acetate
PRP	Pityriasis rubra pilaris
qPCR	Quantitative polymerase chain reaction
SNP	Single nucleotide polymorphism
TPM	Transcripts per million

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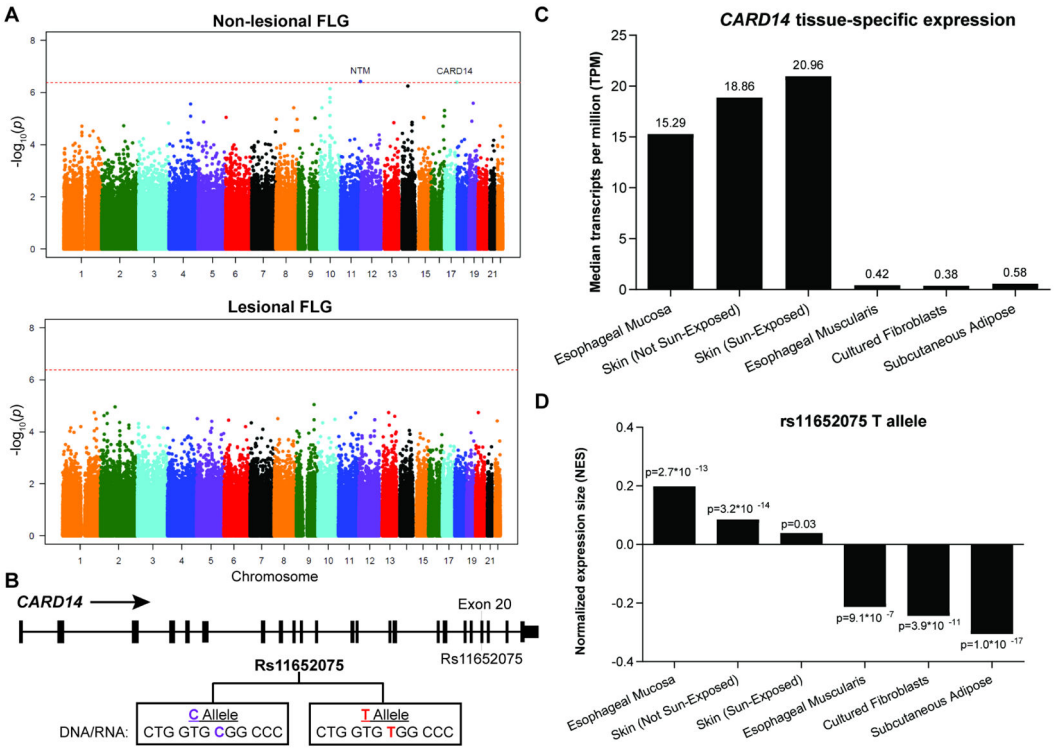

Figure 1. GWAS of *FLG* expression, and *CARD14* is highly expressed in epithelial tissues, where rs11652075 is an eQTL.

A. Manhattan plot of the GWAS of non-lesional and lesional *FLG* in the MPAACH cohort (N = 240). The horizontal dashed red line indicates the preset threshold of $p = 4.2\times10^{-7}$ **B.** Schematic of the location of rs11652075 within the *CARD14* gene and the nucleotide change caused by the rs11652075. **C.** Tissue-specific expression of the *CARD14* gene in median transcripts per million according to the Genomic Tissue Expression (GTEx) database. **D.** The tissue-specific normalized effect size (NES) of rs11652075 on *CARD14* expression according to the GTEx database.

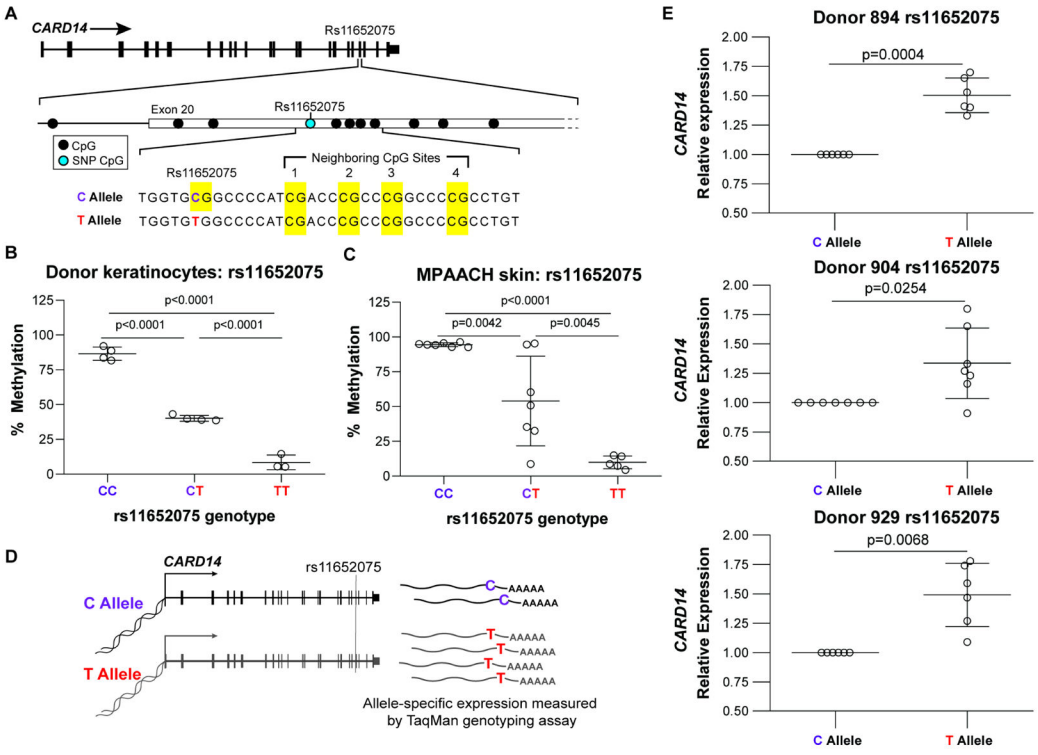

Figure 2. The rs11652075 T-allele abrogates methylation of *CARD14* at the rs11652075 CpG and increases *CARD14* expression in primary human keratinocytes.

A. Schematic of the location of the rs11652075 CpG site and other nearby CpGs. **B-C.** The percent methylation of the rs11652075 CpG site in (**B**) primary human donor keratinocytes and (**C**) human skin from MPAACH subjects of each rs11652075 genotype (CC, CT, TT). Data were analyzed using a one-way ANOVA with Tukey's *post-hoc* tests. Primary keratinocytes: N = 3 (CT, TT) or 4 (CC) donors per genotype. MPAACH skin tapes: N = 5 (TT) or 7 (CC, CT) subjects per genotype. **D.** Schematic of the allele-specific qPCR method. **E.** The relative expression of *CARD14* from the T allele versus C allele determined by allele-specific qPCR in primary keratinocytes from 3 separate donors heterozygous for rs11652075. Data were analyzed using a one-sample two-tailed t-test on the expression ratio using a reference value of 1. N = 6 (donors 894, 929) or 7 (donor 904) per donor. All data are represented as mean ± SD.

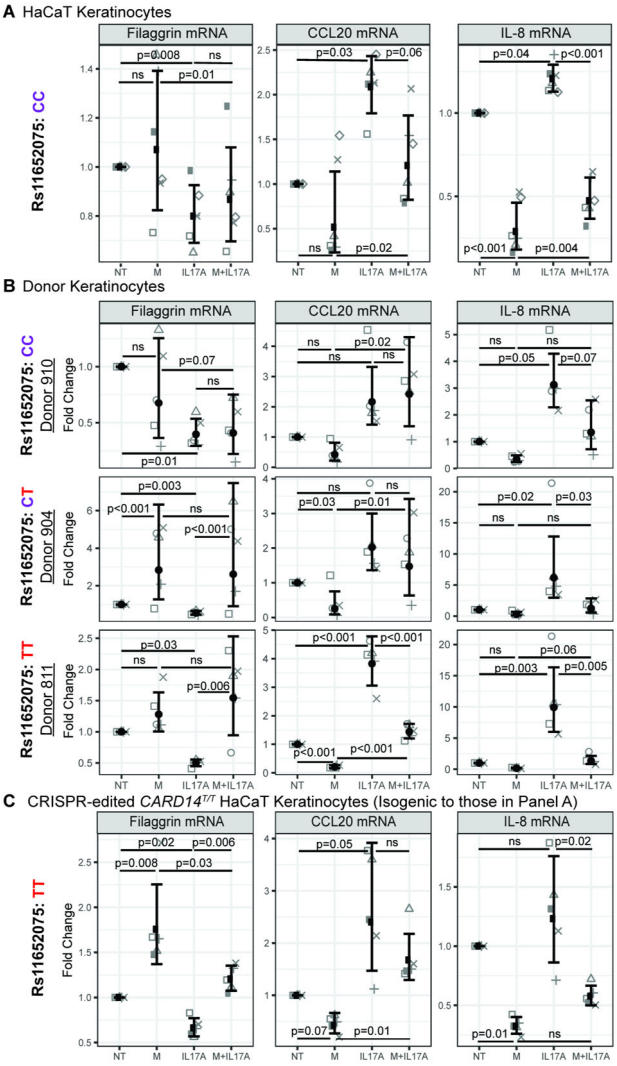

Figure 3. IL-17A-induced *FLG* suppression is rescued by the MALT1 inhibitor mepazine in a rs11652075 genotype-dependent manner.

A. The fold change expression of *FLG, CCL20* and *CXCL8 in CARD14*$^{C/C}$ HaCaT keratinocytes upon IL-17A ± mepazine treatment. N = 6. **B**. Same as (**A**) but in primary keratinocytes of each rs11652075 genotype. N = 5. **C.** Fold change expression of *FLG, CCL20* and *CXCL8* in CRISPR-Cas9-generated *CARD14*$^{T/T}$ HaCaT keratinocytes isogenic to the CC HaCaT keratinocytes in panel A except at rs11652075. N = 6. All data were analyzed on non-normalized ddCt values using repeated-measures two-way ANOVAs with Tukey's *post-hoc* tests. Data are shown normalized to control and are represented as mean ± SD. Symbols designate values from individual replicates. ns = not significant, NT = non-treated, M = mepazine.

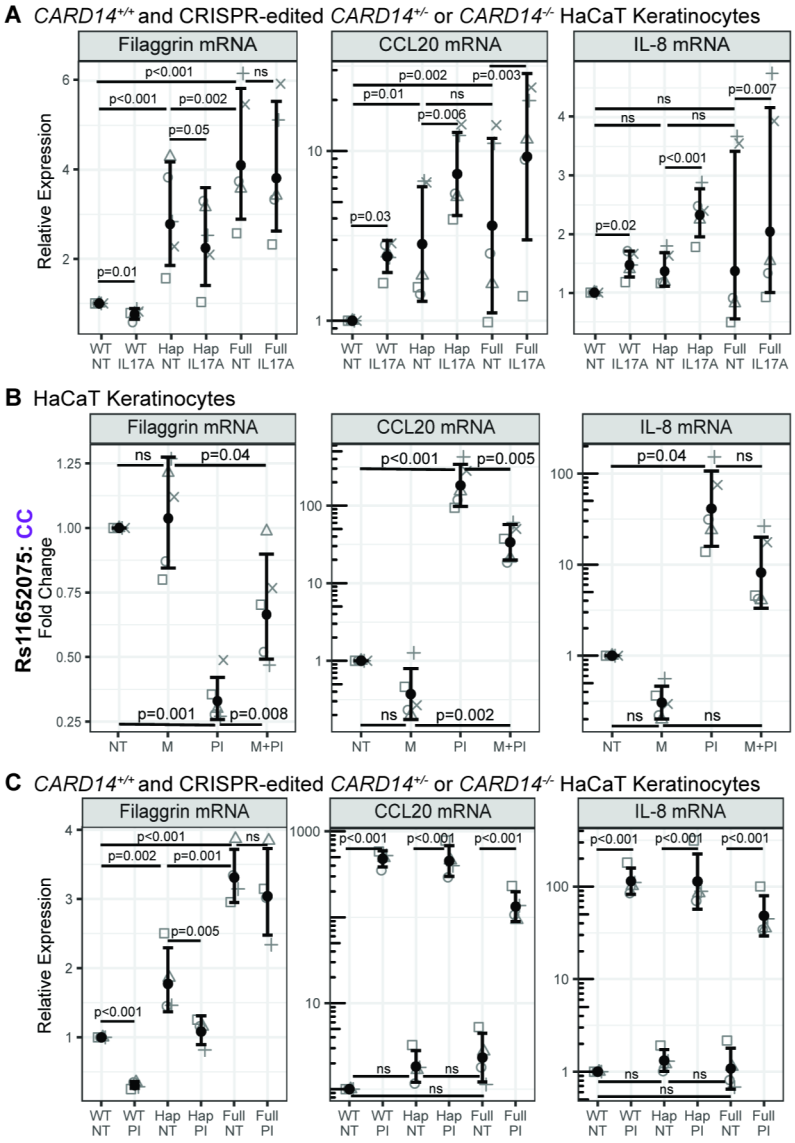

Figure 4. CARD14 deficiency increases baseline *FLG* levels and rescues *FLG* suppression induced by IL-17A and PMA/ionomycin (PI).

A. Relative expression of *FLG, CCL20* and *CXCL8* at baseline and with IL-17A treatment in *CARD14*$^{+/+}$, *CARD14*$^{+/-}$, and *CARD14*$^{-/-}$ *CRISPR*-generated HaCaT keratinocytes isogenic except for rs11652075. N = 5. **B.** Fold change expression in wild-type *CARD14*$^{C/C}$ HaCaT keratinocytes upon 8 hours of PI ± mepazine. N = 5. **C.** Same as (**A**) but with PI treatment. N = 4. All data were analyzed on non-normalized ddCt values using repeated-measures two-way ANOVAs with Tukey's *post-hoc* tests. Data are shown normalized to control and are represented as mean ± SD. Symbols designate values from individual replicates. ns = not significant; NT = non-treated; PI = PMA/ionomycin.

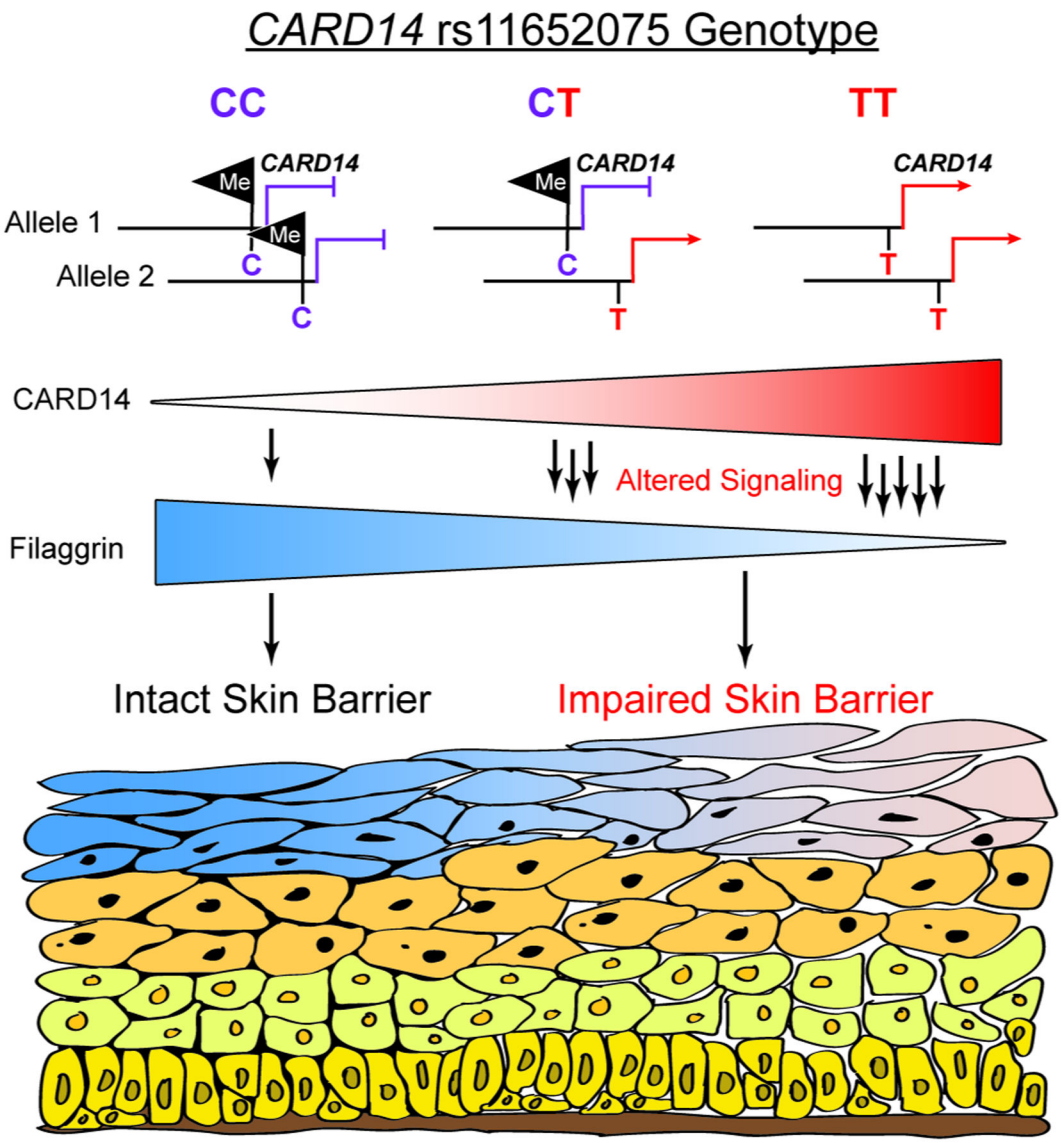

Figure 5. A model for the mechanistic contribution of *CARD14* rs11652075 to reduced *FLG* expression in non-lesional skin.

The T allele prevents methylation at the rs11652075 CpG site and increases *CARD14* expression in heterozygous and homozygous keratinocytes. Since CARD14 regulates *FLG* expression, altered *CARD14* levels and genotype-dependent signaling mechanisms contribute to suppressed *FLG* that may promote to a disrupted barrier phenotype in non-lesional skin. Me = methyl group.