

***Tmem79/Matt* is the matted mouse gene and is a predisposing gene for atopic dermatitis in human subjects**

Sean P. Saunders, PhD,^{a,b,c,*} Christabelle S. M. Goh, BSc,^{d,*} Sara J. Brown, MD,^d Colin N. A. Palmer, PhD,^e Rebecca M. Porter, PhD,^{a,d} Christian Cole, PhD,^{d,f} Linda E. Campbell, MSc,^d Marek Gierlinski, PhD,^f Geoffrey J. Barton, PhD,^f Georg Schneider, PhD,^g Allan Balmain, PhD,^h Alan R. Prescott, PhD,ⁱ Stephan Weidinger, MD, PhD,^j Hansjörg Baurecht, PhD,^j Michael Kabesch, MD,^k Christian Gieger, PhD,^l Young-Ae Lee, MD,^m Roger Tavendale, PhD,^e Somnath Mukhopadhyay, MD,ⁿ Stephen W. Turner, MD,^o Vishnu B. Madhok, MD,^p Frank M. Sullivan, MD,^p Caroline Relton, PhD,^q John Burn, MD,^q Simon Meggitt, MD,^r Catherine H. Smith, MD,^s Michael A. Allen, PhD,^s Jonathan N. W. N. Barker, MD,^s Nick J. Reynolds, MD,^t Heather J. Cordell, DPhil,^q Alan D. Irvine, MD,^{a,b,u} W. H. Irwin McLean, DSc,^d Aileen Sandilands, PhD,^d and Padraic G. Fallon, PhD^{a,b,c} Dublin, Ireland, Dundee, Brighton, Aberdeen, Newcastle, Newcastle-upon-Tyne, and London, United Kingdom, Singapore, San Francisco, Calif, and Kiel, Regensburg, Neuherberg, and Berlin, Germany

Background: Atopic dermatitis (AD) is a major inflammatory condition of the skin caused by inherited skin barrier deficiency, with mutations in the filaggrin gene predisposing to development of AD. Support for barrier deficiency initiating AD came from flaky tail mice, which have a frameshift mutation in *Flg* and also carry an unknown gene, *matted*, causing a matted hair phenotype.

Objective: We sought to identify the *matted* mutant gene in mice and further define whether mutations in the human gene were associated with AD.

Methods: A mouse genetics approach was used to separate the *matted* and *Flg* mutations to produce congenic single-mutant strains for genetic and immunologic analysis. Next-generation sequencing was used to identify the *matted* gene. Five

From ^athe School of Medicine, Trinity College Dublin; ^bthe National Children's Research Centre, Our Lady's Children's Hospital, Dublin; ^cTrinity Biomedical Sciences Institute, Trinity College Dublin; ^dthe Centre for Dermatology and Genetic Medicine; ^ethe Biomedical Research Institute; ^fthe Bioinformatics Research Group; ^gthe Division of Cell Signalling and Immunology, and ^hPopulation Health Sciences, University of Dundee; ⁱthe Bioinformatics Institute, A*STAR, Singapore; ^jthe Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco; ^kthe Department of Dermatology, Allergy, and Venerology, University Hospital Schleswig-Holstein, Kiel; ^lthe Department of Pediatric Pneumology and Allergy, Children's Hospital Regensburg; ^mthe Institute of Genetic Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg; ⁿPediatric Pneumology and Immunology, Charité University Medicine Berlin and Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch, Berlin; ^othe Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and Sussex Medical School, Brighton; ^pthe Department of Child Health, University of Aberdeen; ^qthe Institute of Genetic Medicine, Newcastle University; ^rthe Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne; ^sSt John's Institute of Dermatology, King's College London; ^tthe Institute of Cellular Medicine, Newcastle University; and ^uthe Department of Paediatric Dermatology, Our Lady's Children's Hospital, Crumlin, Dublin.

*These authors contributed equally to this work.

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Corresponding author: Padraic G. Fallon, PhD, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland. E-mail: pfallon@tcd.ie. Or: W. H. Irwin McLean, DSc, Centre for Dermatology and Genetic Medicine, University of Dundee, Dundee, United Kingdom. E-mail: w.h.i.mclean@dundee.ac.uk. 0091-6749/\$36.00

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independently recruited AD case collections were analyzed to define associations between single nucleotide polymorphisms (SNPs) in the human gene and AD.

Results: The matted phenotype in flaky tail mice is due to a mutation in the *Tmem79/Matt* gene, with no expression of the encoded protein mattrin in the skin of mutant mice. *Matt^{ft}* mice spontaneously have dermatitis and atopy caused by a defective skin barrier, with mutant mice having systemic sensitization after cutaneous challenge with house dust mite allergens. Meta-analysis of 4,245 AD cases and 10,558 population-matched control subjects showed that a missense SNP, rs6694514, in the human *MATT* gene has a small but significant association with AD.

Conclusion: In mice mutations in *Matt* cause a defective skin barrier and spontaneous dermatitis and atopy. A common SNP in *MATT* has an association with AD in human subjects. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Allergy, association, atopic dermatitis, atopy, eczema, filaggrin, flaky tail, Matt, mattrin, mouse, mutation, Tmem79

Atopic dermatitis (AD) is the most common diagnosis in dermatology, affecting approximately 1 in 5 children in the developed world,¹ and is frequently associated with atopic asthma and a wide range of allergies.² AD is a highly heritable complex trait; however, environmental influences also play a role in triggering the atopic diathesis.³ Genome-wide association studies in AD have identified several susceptibility loci⁴⁻⁷; however, the major and only functionally characterized genetic factor is the filaggrin gene (*FLG*), which encodes the skin barrier protein filaggrin.⁵ Prevalent loss-of-function variants in *FLG* were identified as the cause of the single-gene disorder ichthyosis vulgaris (dry flaky skin).⁸ Soon thereafter, these variants were shown to be strongly associated with AD,⁹ with heterozygous odds ratios (ORs) of greater than 7 and homozygous ORs of greater than 150 in case-control studies in which both prevalent and rare variants were analyzed.¹⁰

The hypothesis that skin barrier deficiency in the context of *FLG* mutations is an initiator of AD was confirmed experimentally by using the flaky tail mouse mutant,¹¹ which was shown to carry a frameshift mutation in the murine *Flg* gene.¹² Flaky tail mice have a defective skin barrier, with increased percutaneous transfer of antigens and chemical haptens.¹²⁻¹⁵ The *ft* mutation arose spontaneously in 1958 in the progeny of crosses between heterogeneous stocks of mice with the recessive mutation *matted* (*ma*), and these mutations are maintained as a double-mutant (DM) strain known as *maft*. The matted hair phenotype was used for many years as a surrogate marker for the *ft* mutation because, remarkably, the *ft* and *ma* mutations are closely linked on chromosome 3 in the mouse.¹⁶ The DM *maft* mice have been routinely used for studies of skin barrier-deficient AD in recent years.^{13-15,17} In mice and human subjects the *FLG* gene resides in the epidermal differentiation complex, a cluster of more than 70 genes encoding proteins involved in skin barrier formation and differentiation of stratified epithelia,¹⁸ including those within the hair follicle.¹⁸⁻²¹ We suspected the nearby *ma* gene might also be involved in epithelial barrier function, and in this study we set out to separate this allele from *Flg^{ft}* and identify the causative defect.

Abbreviations used

AD:	Atopic dermatitis
DM:	Double mutant
FLG:	Filaggrin
HDM:	House dust mite
hpf:	High-power field
MAPEG:	Membrane-associated proteins in eicosanoid and glutathione metabolism
OR:	Odds ratio
SNP:	Single nucleotide polymorphism
TEWL:	Transepidermal water loss
WT:	Wild-type

METHODS

Isolation of the matted mouse strain

DM *Matt^{ma/ma}Flg^{ft/ft}* mice were provided by Dr John P. Sundberg (Jackson Laboratory, Bar Harbor, Me).¹² DM mice were crossed with C57BL/6J mice to generate *Matt^{ma/+}Flg^{ft/+}* mice. The *Flg^{ft}* and *Matt^{ma}* mutations were separated and backcrossed to congenic C57BL/6J background in accordance with the breeding strategy outlined (see Fig E1 in this article's Online Repository at www.jacionline.org). C57BL/6J mice were used as wild-type (WT) control animals. B6.CBAGr-ma/J (JAX *Matt^{ma/ma}*) mice were obtained from the Jackson Laboratory. Mice were housed in specific pathogen-free conditions, with irradiated diet and bedding and water *ad libitum*. All animal experiments were performed in compliance with Irish Department of Health and Children regulations and approved by Trinity College Dublin's BioResources Ethical Review Board.

Gene mapping

Skin samples were obtained from neonatal mice, and DNA was extracted by using the DNA Purification Kit (Promega, Madison, Wis). Genomic DNA extracted from murine neonatal blood was amplified with the GoTaq Flexi DNA Polymerase kit (Promega). All samples were sequenced by using the ABI 3730 DNA Systems (Applied Biosystems, Foster City, Calif). Mapping primers were used to amplify and sequence murine chromosome 3 (see Table E1, A, in this article's Online Repository at www.jacionline.org). Matted genomic sequences were compared with C57BL/6J for regions of congenicity.

Next-generation sequencing and bioinformatics

Three replicates from each sample (WT and *Matt^{ma/ma}*) were submitted for next-generation sequencing. The replicates were run multiplexed on an Illumina GAIIx and HiSeq 2000 (Illumina, San Diego, Calif) by using v3 sequencing chemistry and the Roche 454 Titanium workflow (Roche, Mannheim, Germany). For further details on sequencing, bioinformatics, and single nucleotide polymorphism (SNP) and InDel methodologies, see the Methods section in this article's Online Repository at www.jacionline.org.

Analysis and identification of murine *Tmem79/Matt* gene

Each of the 4 exons was amplified individually by using PCR with the following conditions for all exons: 1 cycle of 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute; and a final extension at 72°C for 5 minutes.

Semiquantitative RT-PCR

Mouse tissue samples were lysed with TissueLyser LT (Qiagen, Hilden, Germany), and RNA was extracted with the RNeasy kit (Qiagen). RNA was reverse transcribed with the ImProm-II Reverse Transcription System (Promega). An intron-spanning amplification was carried out on *Tmem79/Matt* across exons 3 and 4. *Krt14* was used as a loading control. RT-PCR primers used are shown in Table E1, B, in this article's Online Repository.

Mouse genotyping

The 644-bp PCR product of the third *Tmem79/Matt* exon was digested with the restriction enzyme CviQI (New England Biolabs, Ipswich, Mass), and digested fragments were separated on agarose gel by using electrophoresis. Primers used are shown in Table E1, A. The *Flg^{fl}* mutation was genotyped, as previously described.¹²

Immunoblotting

The epidermis was separated from the dermis of neonatal mice after immersion in 5 mmol/L EDTA at 50°C for 5 minutes, followed by cooling in ice-cold PBS. The separated epidermis was extracted in urea/Tris buffer containing protease inhibitor cocktail (Halt; Thermo Scientific, Erembodegem, Belgium) by means of homogenization. Protein samples were separated on SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Temecula, Calif), which were probed with rabbit polyclonal antibodies specific for human mattrin (TMEM79; Novus Biologicals, Littleton, Colo). Primary antibodies were detected by means of incubation with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Dako, Stockport, United Kingdom). Immunolabeled proteins were visualized by using chemiluminescence with the ECL detection system (Millipore).

Immunofluorescence and Nile Red staining

Dorsal murine sections were obtained from 4-day-old neonatal mice and snap-frozen immediately. All frozen samples were then cryosectioned at 4 to 5 μ m and stored at 80°C until use. After drying, sections were fixed in 50% methanol acetone, washed in PBS, and incubated with primary antibodies. Nuclei were stained with 4'-6-diamidino-2-phenylindole (Sigma-Aldrich, St Louis, Mo), and slides were fixed in Hydromount (National Diagnostics, Atlanta, Ga). Primary antibodies used in whole mounts were as follows: polyclonal TMEM79 (TMEM79; Novus Biologicals) and monoclonal CK-13 against cytokeratin 17 (Sigma-Aldrich). Frozen mouse skin sections were stained with Nile Red and 6-diamidino-2-phenylindole. Images were collected with a Zeiss LSM700 confocal microscope (Zeiss, Oberkochen, Germany).

Quantitative PCR of human cDNA tissue array

Expression of *TMEM79/MATT* cDNA was quantified across a multiple-tissue panel containing cDNA from 48 different human tissues (Origene Technologies, Rockville, Md). This was done with the TaqMan Gene Expression Assay, C_25986870_10 (Life Technologies, Carlsbad, Calif), which spans exons 3 to 4 to result in amplicon length of 65 bp in cDNA sequence. The standard used in this assay was a commercially available plasmid containing the full-length WT *TMEM79/MATT* (Origene Technologies). A standard curve was calculated by using the formulae provided by Applied Biosystems.

Phenotypic scoring

The severity of inflammation and AD-like pathology was scored by using the macroscopic diagnostic criteria described for the skin inflammation seen in the Nc/Nga mouse model of AD.²² Briefly, a scoring system (0, none; 1, mild; 2, moderate; and 3, severe) was applied to the symptoms of pruritus, edema, erosion, scaling, and erythema. Pruritus (scratch behavior) was observed for 5 minutes. Total scores for each mouse were calculated from the sum of individual scores.

Cutaneous challenge with house dust mite antigen

Mice were challenged with house dust mite (HDM) to induce allergic skin sensitization, as previously described.¹³ Briefly, HDM (Greer Laboratories, Lenoir, NC) was topically applied to intact skin of WT and *Matt^{ma}* mice by using Finn chamber patch tests (SmartPractice, Phoenix, Ariz) and secured with Scanpor tape (Bio-Diagnostics, Worcester, United Kingdom). The regimen involved five 1-week exposures to the allergen patch separated by 2-week intervals. Hair was clipped 24 hours before application of HDM that was prepared in endotoxin-free Dulbecco PBS and vehicle to a concentration of 1 mg/mL. Dulbecco PBS was used as the control. Mice were assessed 24 hours after the final HDM challenge.

ELISA

Serum levels of HDM-specific IgE, IgG₁, and IgG_{2a} were detected by means of direct ELISA. Total serum IgE levels were measured with a sandwich ELISA, according to the manufacturer's instructions (BD PharMingen, San Jose, Calif).

Histology

Dorsal skin sections were removed and fixed in 10% formal saline. Paraffin-embedded sections were stained with hematoxylin and eosin and examined in blinded fashion by 2 observers independently. To quantify dermal cell numbers, we used a previously described scoring system.¹² The total number of cells per high-power field (hpf) of view were counted on 20 hpfs per mouse. For skin sections, an arbitrary histologic scoring system was used to quantify acanthosis and hyperkeratosis. Acanthosis scoring was based on the magnitude of epidermal hyperplasia, and hyperkeratosis scoring was based on the magnitude of stratum corneum thickening, with scores (0, basal; 1, mild; 2, moderate; 3, marked; 4, very marked; and 5, extreme pathology) for each parameter based on measurements per hpf in sections.

Measurement of transepidermal water loss

A Courage and Khazaka Tewameter TM210 (Enviroderm, Evesham, United Kingdom) was used for measurement of transepidermal water loss (TEWL), which was measured on the dorsal flank of mice 24 hours after hair clipping. TEWL was recorded at an ambient temperature of 19°C to 21°C and humidity of 50% $\pm$ 5%. In mice sensitized to HDM, TEWL was measured in PBS- and HDM-treated mice before and after the HDM challenge regimen.

Electron microscopy

For scanning electron microscopy, hair fibers were removed from mice and fixed in cacodylate-buffered glutaraldehyde. Samples were prepared for scanning electron microscopy by using established procedures¹¹ and examined in a Zeiss Supra scanning electron microscope.

Statistical analysis of mouse studies

GraphPad Prism software (GraphPad Software, La Jolla, Calif) was used for data analysis. Differences between groups were determined by using the Student *t* test and 2-way ANOVA. Results are presented as means $\pm$ SEMs. Differences, indicated as 2-tailed *P* values, were considered significant at a *P* value of less than .05.

SNP identification in human subjects

Human samples from England, Scotland, and Ireland were obtained with consent from patients and Institutional Ethics Committee approval complying with the principles of the Declaration of Helsinki. Human *MATT* was sequenced by using primers listed in Table E1, C, in this article's Online Repository at www.jacionline.org. Conditions for all exons except exon 2.2 were as follows: 1 cycle of 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute; and a final extension at 72°C for 5 minutes. An annealing temperature of 58°C was used for exon 2.2. SNP rs6684514 (see Table E2 in this article's Online Repository at www.jacionline.org) was genotyped across the case-control study populations by using the TaqMan allelic discrimination genotyping assay (C_25986870_10, Life Technologies Corporation).

Case-control studies

Five independently recruited AD case collections were compared with ethnically matched population control subjects (see the **Methods** section in this article's Online Repository). Case definitions are described in the **Methods** section in this article's Online Repository, and demographic data relating to these AD case collections and control subjects are summarized in Table E3 in this article's Online Repository at www.jacionline.org.

Genotype imputation

For *in silico* replication, we used the 2 German AD case-control selections (see Table E3), which had been genotyped on Illumina 300k and Affymetrix

6.0p (Affymetrix, Santa Clara, Calif), respectively, and imputed based on the 1000 Genomes database (phase I integrated variant set release, March 2012). Previous imputation data sets were curated, and samples with a greater than 5% missing rate, excess of heterozygosity (± 5 SDs away from the sample mean), and unexpected relatedness ($PI_{HAT} > 0.1875$, halfway between expected IBD for third- and second-degree relatives) were excluded. Further outliers from the multidimensional scaling of the pairwise IBS matrix were removed. Imputation was carried out with SHAPEIT and IMPUTE2. The SNP rs6684514 investigated *in silico* had an imputation quality of 0.996 (proper info) and a CR of 0.997.

Statistical genetics

Case-control comparisons for each case collection and population control subjects were performed by using logistic regression analysis in Stata 12.0 (StataCorp, College Station, Tex). Meta-analysis (under fixed- and random-effects models) of the resulting log ORs and their SEs was performed with the “metan” function in Stata 12.0.

RESULTS

Identification of *Matt* as the gene mutation in matted mice

To separate the 2 mutations in the DM *maft* mice, we used the backcross-intercross protocol outlined in Fig E1. Mice with the *Flg^{fl}* mutant allele (*Flg* c.5303delA) were genotyped, as previously described.¹² Progeny from putative heterozygous intercrosses were checked for the recessive matted hair phenotype to identify *ma/ma* homozygotes. By this means, we separated both alleles. Because SNP analysis showed that the initial DM *maft* mouse was maintained on a mixed-strain background, we generated single-mutant N12 mice congenic on the C57BL/6J background. By using this backcross-intercross strategy to generate congenic *ma/ma* homozygous mice (see Fig E1), the recessive disease-causing locus should be ultimately visible as a small area of homozygosity flanked by 2 unequal heterozygous regions (see Fig E2, A, in this article's Online Repository at www.jacionline.org). To map this, we sequenced several short PCR fragments across the region from C57BL/6J congenic *ma/ma* homozygotes (see Table E1, A) to identify SNPs. By this means, a 1.05-Mb critical interval was defined (Fig 1, A). In parallel, we used epidermal transcriptomic sequencing to compare WT C57BL/6J and congenic *ma/ma* homozygotes. A nonsense mutation, p.Y280*, in the *Tmem79* gene, located within the interval (Fig 1, A), was identified in the RNAseq data (not shown). The mutation in *Tmem79* was confirmed by using conventional sequencing of genomic PCR products (Fig 1, B) and restriction digests (see Fig E2, B). This mutant transcript was expressed at approximately 30% of the WT levels in *ma/ma* epidermis (Fig 1, C). By using a commercially available antibody against the C-terminus of the encoded protein, a single band of approximately 49 kDa was seen in epidermal extracts from WT and congenic *Flg^{fl/fl}* mice, with the protein absent in congenic *ma/ma* and DM mice (Fig 1, D), indicating the presence of a truncated protein or an absence of protein in these mice. Interestingly, epidermal extracts from *Flg^{fl/fl}* mice had increased expression of protein relative to WT extracts. *Tmem79* was renamed *Matt*, encoding the protein mattrin; similarly, the human ortholog was renamed *MATT* (approved by the Genome Nomenclature Committee). By convention, after identification of the gene, the *ma* mutant allele was redesignated as *Matt^{ma}*.

Mattrin is expressed in mouse and human skin

Matt is a compact 5-exon gene on mouse 3qF1 spanning 5781 bp of genomic DNA, encoding a 391-amino-acid protein with a calculated molecular weight of 43.5 kDa. The human ortholog, *MATT*, which is syntenic on 1q23.1, has an identical organization. Mattrin is a transmembrane domain protein with no previously ascribed function (Fig 1, E). Bioinformatics analysis predicts a long internal N-terminal domain, 5 transmembrane domains, and a short C-terminus (Fig 1, E, and see Fig E3 in this article's Online Repository at www.jacionline.org). The mutation p.Y280* occurs before the third transmembrane domain (Fig 1, E). Immunohistochemistry revealed that mattrin was present in epidermal granular layer keratinocytes in WT mice (Fig 2, A) but was absent from *Matt^{ma/ma}* animals (Fig 2, B). Similarly, the protein was observed in granular layer cells in human epidermis (see Fig E4, A and B, in this article's Online Repository at www.jacionline.org) and was also found in the hair follicles of mice (Fig 2, A) and human subjects (see Fig E4, C and D). In addition, the mRNA is widely expressed in human tissues and, consistent with immunohistochemistry, is strongly expressed in skin (see Fig E4, E).

Expression quantitative trait locus analysis²³ showed that *Matt* is in a network of proteins expressed late in epidermal differentiation, with an expression quantitative trait locus profile closely matching that of *Rhbg* (transporter protein) and *Rab25* (membrane trafficking, data not shown). Mattrin shows distant sequence homology to the Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG) protein family (see Fig E3),²⁴ members of which have roles in lipid catabolism.²⁵ Immunohistochemistry with the lipophilic dye Nile Red revealed highly organized stacks of cornified cell envelopes in the stratum corneum of WT animals (Fig 2, C) that were highly disorganized in *Matt^{ma/ma}* mice, with discontinuous, uneven, and highly disorganized cornified cell envelopes (Fig 2, D).

Matt^{ma/ma} mice have spontaneous dermatitis

Gross examination of adult *Flg^{fl/fl}* and *Matt^{ma/ma}* mice demonstrates that the 2 strains differ considerably from each other (Fig 3, A). Macroscopic clinical scoring²² demonstrated *Matt^{ma/ma}* and DM mice spontaneously having progressive dermatitis-like skin inflammation, which did not occur in adult *Flg^{fl/fl}* animals (see Fig E5, A, in this article's Online Repository at www.jacionline.org). Although all *Matt^{ma/ma}* and DM mice had marked skin inflammation, there was a broad spectrum of pathology, with some animals exhibiting profound lesions and excoriation and occasional blepharitis and eyelid dermatitis (see Fig E6, B, in this article's Online Repository at www.jacionline.org). Interestingly, both neonatal *Flg^{fl/fl}* and DM mice have significant ichthyosis ($P < .001$) compared with WT mice, but *Matt^{ma/ma}* mice did not, indicating the postnatal importance of filaggrin (see Fig E5, A, and E6, A).

With respect to the matted hair phenotype,^{11,26} both *Matt^{ma/ma}* and DM animals had the keratinization defect, with hairs forming clumps^{11,26} and hair breakage and occasional alopecia evident at 32 weeks (Fig 3, A, and see Fig E6, B). Scanning electron microscopy confirmed *Matt^{ma/ma}* and DM mice had fragile hairs prone to longitudinal splitting and breakage with defective cuticle morphology (see Fig E7, A, in this article's Online Repository at www.jacionline.org). Furthermore, *Matt^{ma/ma}* mice had distorted hair follicle morphogenesis (see Fig E7, B).

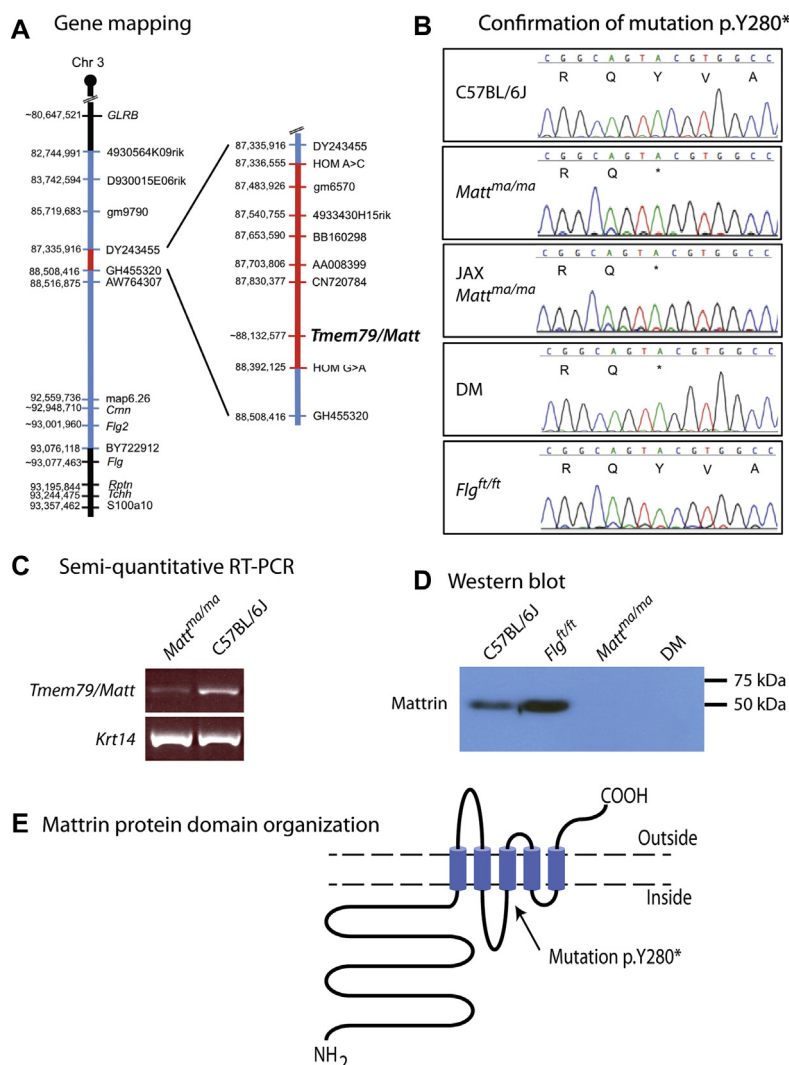


FIG 1. Identification of *Tmem79/Matt* as the *matted* gene. **A**, A region of homozygous *ma/ma* on chromosome 3 indicated by regions in red and heterozygous regions in blue, with the homozygous C57BL/6J genome shown in black. **B**, The *ma* mutation in *Tmem79/Matt* identified by means of epidermal transcriptome sequencing was confirmed by using Sanger sequencing, causing a C to G substitution at position 88,136,485 on chromosome 3. This predicts the protein change p.Y280X in the *Tmem79* (matrin) protein. The mutant allele was renamed *Matt^{ma}*. **C**, Semiquantitative RT-PCR of epidermal mRNA showed a reduction in expression of *Matt* in *Matt^{ma/ma}* animals. **D**, Matrin detected in immunoblotting of skin from WT and *Flg^{fl/fl}* mice but no protein expression in *Matt^{ma/ma}* and DM mice. **E**, Bioinformatics shows matrin consists of 5 transmembrane helices with a long intracellular N-terminus and short extracellular C-terminus.

Skin histology (Fig 3, B) showed that *Matt^{ma/ma}* and DM mice have marked acanthosis ($P < .001$) and prominent orthokeratosis ($P < .001$) with dermal inflammatory infiltrates ($P < .001$; see Fig E5, B). In contrast, *Flg^{fl/fl}* mice had occasional foci of acanthosis, mild diffuse orthokeratosis, and increased dermal cell infiltration relative to WT mice ($P < .001$). However, the phenotype in *Flg^{fl/fl}* mice was subclinical, with no overt dermatitis (Fig 3, A, and see Fig E5, A).

***Matt^{ma/ma}* mutant mice are atopic and have a defective skin barrier**

All mutant strains spontaneously had increased IgE levels relative to WT mice, which was markedly more pronounced in

Matt^{ma/ma} and DM animals relative to *Flg^{fl/fl}* mice ($P < .001$; see Fig E5, D). Because the severity of skin inflammation in AD parallels barrier permeability,^{27,28} TEWL was analyzed to quantify skin barrier dysregulation in mouse strains. TEWL was significantly increased in adult *Matt^{ma/ma}* and DM mice ($P < .001$; see Fig E5, C). In contrast, TEWL was unaltered in *Flg^{fl/fl}* mice relative to that seen in WT animals (see Fig E5, C).

HDM allergen was applied to the intact skin of *Matt^{ma/ma}* and WT mice to address whether the altered skin barrier in *Matt^{ma/ma}* mice influences allergen sensitization. Percutaneous sensitization with allergen led to enhanced skin inflammation in *Matt^{ma/ma}* mice (Fig 4, A-C). Consistent with the defective barrier, TEWL was significantly upregulated ($P < .001$) in HDM-treated *Matt^{ma/ma}* mice after allergen application (Fig 4, D). Crucially,

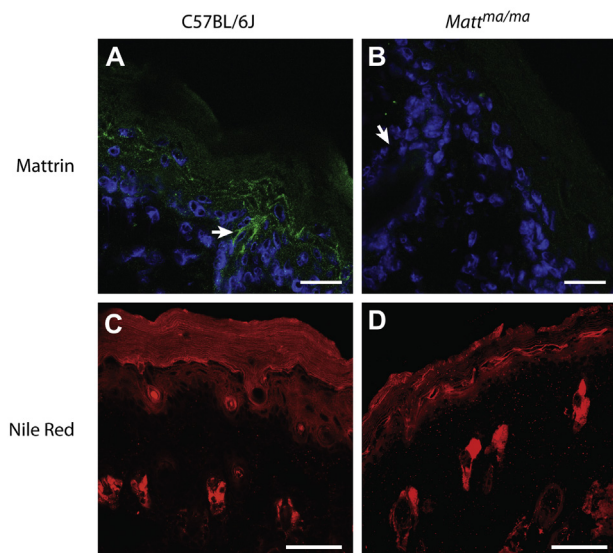


FIG 2. Matrin expression and disorganized lipid morphology in the epidermis of *Matt^{ma}* mice. **A** and **B**, Immunohistochemistry detection of matrin expression confined to the epidermal granular layer of WT (Fig 2, **A**) but not *Matt^{ma/ma}* (Fig 2, **B**) animals, including hair follicles (arrow). Scale bar = 20 μ m. **C** and **D**, Nile Red staining of lipids in the epidermis of WT (Fig 2, **C**) and *Matt^{ma/ma}* (Fig 2, **D**) mice revealed uneven and highly disorganized cornified cell envelopes in the stratum corneum of *Matt^{ma/ma}* mice. Scale bar = 50 μ m.

HDM-specific IgE responses were significantly ($P < .05$) evoked in the sera of HDM-treated *Matt^{ma/ma}* mice, as well as HDM-specific IgG₁ and IgG_{2a} responses (Fig 4, **E**). In contrast, the skin of WT (Fig 4) and *Flg^{fl/fl}* (data not shown) mice was refractory to cutaneous HDM exposure. Collectively, these data demonstrate that *Matt^{ma/ma}* mice have spontaneous AD and a defective skin barrier that facilitates percutaneous allergic sensitization.

A mutation in *MATT* is associated with AD

To investigate whether *MATT* is associated with human AD, all exons of human *MATT* were sequenced in 55 Irish AD cases who were negative for *FLG* null mutations. Several noncoding SNPs and 1 known missense SNP, rs6684514, were identified (see Table E2 in this article's Online Repository at www.jacionline.org). Case-control analyses conducted on 2 independent AD case collections, English adult AD and United Kingdom pediatric AD, with separate population-matched control subjects (see Table E3) showed a significant association between rs6684514 and AD (Table I). The minor allele (A) is protective for disease in both the English adult AD (OR, approximately 0.791; $P = .0038$) and UK pediatric AD (OR, approximately 0.770; $P = .0143$) populations (Table I). Notably, when controlling for the strong and significant *FLG* null mutations, it was shown that the association of rs6684514 with AD is independent of *FLG* (Table I and see Table E4 in this article's Online Repository at www.jacionline.org). The association of rs6684514 with AD was further replicated in a German AD case-control analysis in which the OR after controlling for *FLG* mutations was 0.86 (95% CI, 0.76-0.97; $P = .0161$; Table I). However, this association was not replicated in Irish or Scottish case-control analyses (Table I). A meta-analysis using a fixed-effects model of all 4,245 AD cases from

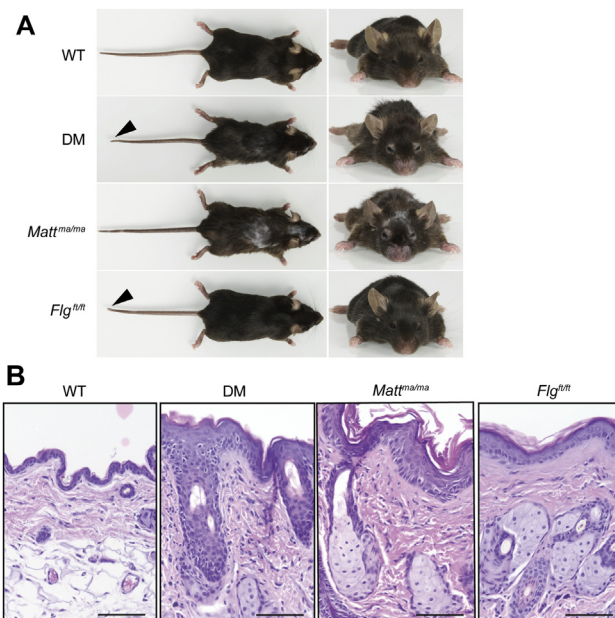


FIG 3. *Matt^{ma}* mice are phenotypically distinct from *Flg^{fl/fl}* mice, with histopathology demonstrating enhanced cutaneous inflammation at 32 weeks. **A**, Gross phenotype of WT, DM, *Matt^{ma/ma}*, and *Flg^{fl/fl}* mice. *Flg^{fl/fl}* mice are indistinguishable from WT mice, apart from the stubbed tail and shortened ear pinnae, which are phenotypic features shared with the DM mouse. All mice are age-matched homozygous males. **B**, Representative skin biopsy specimens from WT, DM, *Matt^{ma/ma}*, and *Flg^{fl/fl}* mice at 32 weeks, showing markedly increased cutaneous inflammation in DM and *Matt^{ma/ma}* mice in comparison with that seen in WT and *Flg^{fl/fl}* mice. Scale bar = 50 μ m.

England, Scotland, Ireland, and Germany with 10,558 population-matched control subjects did not show significant heterogeneity between the study groups ($P = .078$). A small but significant effect of rs6684514 on AD risk was confirmed in the meta-analysis (OR, 0.91; 95% CI, 0.86-0.96; $P = .001$; Fig 5; see Table E4). A random-effects meta-analysis also produced similar results (OR, 0.90; 95% CI, 0.82-0.98; $P = .015$; see Fig E8 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Previously, we demonstrated that *FLG* loss-of-function mutations have a very strong relevance for the common inflammatory skin disease AD and associated atopic phenotypes^{3,9,10} and identified an analogous mutation in the murine homolog *Flg* in the spontaneously occurring flaky tail DM (*maft*) mouse.¹² Subsequently, these DM mice have been widely used as a model of heritable skin barrier deficiency and spontaneous dermatitis^{13-15,17} and as a model of filaggrin deficiency-associated AD pathogenesis in patients.²⁹ In this study we have identified the *matted* phenotype in the DM mouse as arising from a second mutation in *Matt* (*Tmem79*). The *Matt^{ma}* mutation results in defective expression of the transmembrane protein matrin, which is highly expressed in the upper granular layer of epidermal keratinocytes, with a predicted role in lipid homeostasis. These studies have elucidated the relative contributions of the *Flg* and *Matt* mutations in the DM mouse. The *Matt^{ma}* mutation led to the striking development of spontaneous AD-like skin pathology and atopy in adult mice. These data indicate that the DM mouse is not a true model of filaggrin deficiency-associated AD-like skin

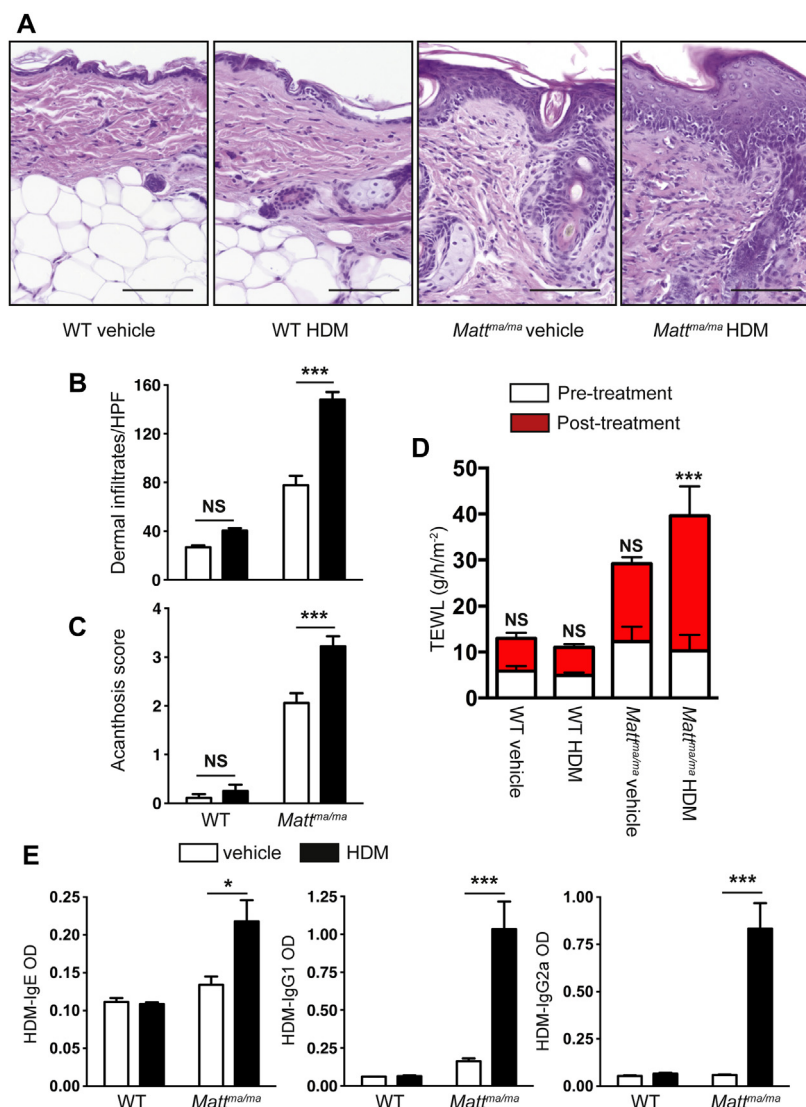


FIG 4. *Matt^{ma}* mice have exacerbated AD-like inflammation and HDM-specific responses after allergen challenge to intact skin. **A**, Representative skin biopsy specimens from age-matched WT and *Matt^{ma/ma}* mice treated with HDM or vehicle, with increased inflammation in HDM-treated *Matt^{ma/ma}* mice relative to that seen in HDM-treated WT and vehicle-treated *Matt^{ma/ma}* mice. Scale bar = 50 μ m. **B**, HDM treatment induces increased dermal cell infiltration in *Matt^{ma/ma}* mice relative to that seen in HDM-treated WT and vehicle-treated *Matt^{ma/ma}* mice. **C**, Acanthosis is increased in HDM-treated *Matt^{ma/ma}* mice relative to that seen in HDM-treated WT and vehicle-treated *Matt^{ma/ma}* mice. **D**, Epicutaneous HDM treatment results in significantly increased TEWL in *Matt^{ma/ma}* mice relative to that seen in HDM-treated WT mice. **E**, HDM-treated *Matt^{ma/ma}* mice have increased HDM-specific serum IgE, IgG₁, and IgG_{2a} levels relative to those seen in HDM-treated WT mice. Cell numbers and acanthosis were scored on 15 to 20 hpfs ($\times 1000$ magnification) on hematoxylin and eosin-stained sections from WT, DM, *Matt^{ma/ma}*, and *Flg^{fl/fl}* mice. Data represent the mean and error bars represent $\pm$ SEMs from 6 to 8 mice and are representative of 2 separate experiments. The Student *t* test or 2-way ANOVA was used to determine statistical differences between groups. NS, Not significant, **P* > .05. ****P* < .001.

inflammation. It is notable that the *Flg^{fl/fl}* mutation has a neonatal influence, whereas the *Matt^{ma}* mutation has a progressive influence with age. This indicates the polygenic nature of the DM mouse as an AD model and indicates the differential influence of both the *Flg^{fl/fl}* and *Matt^{ma}* mutations. Recently, *Flg^{-/-}* mice were shown to have no overt dermatitis with age,³⁰ with the authors unable to sensitize *Flg^{-/-}* mice by means of application of OVA, an allergen commonly used in models of skin inflammation,³¹ to the intact skin barrier, results similar to our data.

Notably, *Flg^{fl/fl}* mice were not sensitized to HDM (data not shown), indicating an impermissibility of the *Flg^{fl/fl}* skin barrier to protein antigen ingress. These data indicate that the DM mouse is not a true model of filaggrin deficiency-associated AD-like skin inflammation.

In this study we adopted a translational approach and also addressed whether mattrin was implicated in human AD. A missense SNP in *MATT* was shown to have a small but significant effect on the risk for human AD. Because mattrin shows

TABLE I. Results of case-control analyses to investigate the association of rs6684514 and AD in 5 populations

Case-control comparison	Cases (n)	Control subjects (n)	P value for rs6684514	OR	95% CI	P value for rs6684514 association after controlling for <i>FLG</i> null mutations
English adult severe AD vs English population control subjects from the 1958 Birth Cohort	505	1919	.0038	0.791	0.674-0.929	.0008
UK mild-moderate pediatric AD vs English pediatric control subjects without AD	338	538	.0153	0.770	0.622-0.953	.0328
Irish pediatric AD vs Irish adult population control subjects	724	1905	.1300	1.025	0.896-1.172	.7905
German AD cases vs German population control subjects	1543	2005	.0794	0.912	0.822-1.011	.0161
Scottish asthma cases with AD vs Scottish population control subjects	1135	4189	.1365	0.922	0.831-1.023	NA

English adult severe AD is defined as early-onset, persistent, and severe disease. *UK mild-moderate pediatric AD* includes cases collected from an English population birth cohort ($n = 177$) and a Scottish General Practice collection ($n = 161$); the *English pediatric control subjects* were ascertained not to have AD at the age of 7 to 9 years. *Irish pediatric AD* cases were collected in secondary and tertiary care clinics in Ireland; *Irish population control subjects* are healthy adult blood donors. *Scottish asthma cases with AD* are subjects with physician-diagnosed asthma and parent-reported AD. The German case and control rs6684514 genotypes were ascertained by imputation from genome-wide SNP analysis data; all other SNP genotypes and *FLG* null mutations were analyzed by using TaqMan allelic discrimination assays. In the English, United Kingdom, and Irish collections, 4 prevalent *FLG* null mutations were analyzed (R501X, 2282del4, R2447X, and S3247X), whereas in the German population 2 *FLG* null mutations were analyzed (R501X and 2282del4). Statistical analysis was performed through logistic regression in Stata 10.0 software (StataCorp).

NA, *FLG* genotype data not available.

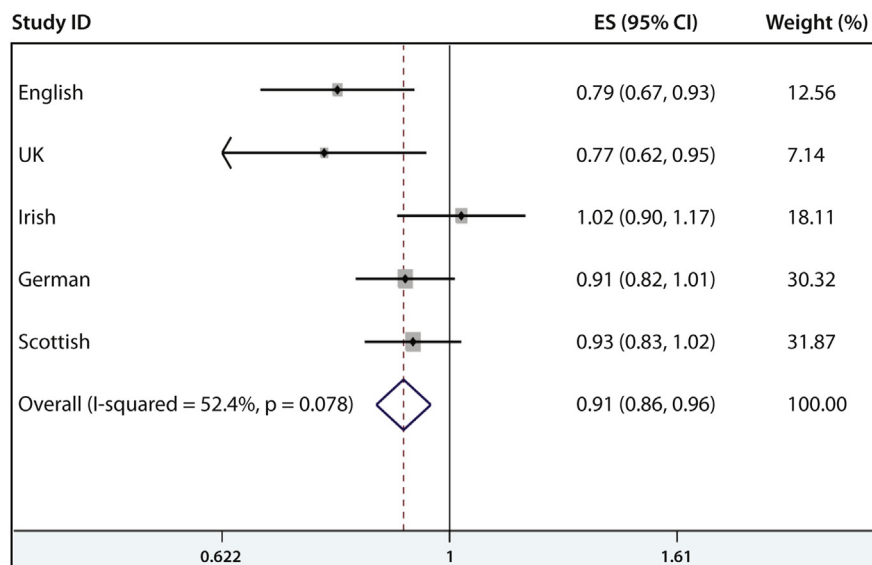


FIG 5. Forrest plot showing results of a fixed-effects meta-analysis of 5 case-control studies to investigate the association of rs6684514 and AD. Study populations used were as follows: *English*, English adult severe AD versus English population control subjects from the 1958 Birth Cohort; *UK*, UK mild-moderate pediatric AD versus English pediatric control subjects without AD; *Irish*, Irish pediatric AD versus Irish adult population controls; *German*, German AD cases versus German population control subjects; *Scottish*, Scottish asthma cases with AD versus Scottish population control subjects. *ES*, Estimated odds ratio. Meta-analysis was carried out with the “metan” function in Stata software (StataCorp).

distant sequence homology to MAPEG family members,²⁴ which are known to catalyze glutathione-dependent transformations of lipophilic substrates at the lipid bilayer, sequence homology suggests a possible role for mattrin in the biology of lipids or lipid-like molecules.²⁵ Epidermal transcriptomic analysis of WT versus *Matt^{ma/ma}* mice has revealed changes in several transcripts involved in fatty acid and lipid biology (data not shown). However, 1-dimensional and 2-dimensional thin-layer chromatography of epidermal lipid extracts did not reveal any differences in the migration or abundance of ceramides, phospholipids, or polar or nonpolar lipids between WT and *Matt^{ma/ma}* mice (data not shown). Future work will require analysis of the glutathione

binding of mattrin, elucidation of lipophilic substrates of mattrin, and an investigation of the downstream signaling pathways and local immunologic milieu in *Matt^{ma}* mice.

In summary, using a translational mouse-patient strategy, we have identified a new gene mutation that leads to dermatitis in mice and have further demonstrated that a variant in the human gene is associated with AD.

We thank the patients affected by eczema and their families for their interest and participation. We also thank Masa Amagai and his research group from Keio University, Tokyo, Japan, for their open and enthusiastic collaboration, discussion, and sharing of data.

Clinical implications: The role of *MATT* mutations in susceptibility to AD and related allergic conditions should be further investigated.

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ONLINE REPOSITORY

METHODS

Next Generation Sequencing and Bioinformatics

The raw fastq data provided had between 67 M – 105 M 76 bp paired-end reads per replicate. Fastqc (v0.10.0 <http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/>) was used to check their read quality before alignment to the Ensembl 66 mouse genome (1) with the BWA short read aligner (2) (v0.5.9-r16 setting fastq format to Illumina v1.3+, parameter ‘-I’). Read counts were assigned to genes with HTSeqcount:

<http://www.huber.embl.de/users/anders/HTSeq/doc/count.html> using the following parameters: mode=union; type=exon; idattr=gene_id.

The Ensembl 66 GTF file was used for gene annotations, resulting in 28,007 genes expressed in at least one of the replicates. Differential gene expression between the WT and the Matted samples was performed with DESeq (v1.6.1) (3) and R (v2.14.0) with significant genes defined as those with $p < 0.01$. In order to concentrate on genes with the most reliable read counts, genes that had <120 reads in total (i.e. on average <20 reads per sample) across all six samples were removed, leaving 18,078 genes.

SNP and InDel Detection Methodology

All replicates were combined into one BAM file per sample and the aligned reads were passed through SAMtools v0.1.18 (4) for calling SNPs and InDels with the standard command: samtools mpileup -C50 -DS -uf <ref> <bams> | bcftools view -

bvcg > raw.bcf. The called SNPs/InDels were filtered to report only those with a minimum read depth of 20. SNPs/InDels were compared between the WT and *Matt^{ma/ma}* samples and only those found in the *Matt^{ma/ma}* samples were retained for further analysis. 383 SNPs and InDels were identified in the EDC region (chromosome 3: 87,000,000-95,000,000) as being unique to the *Matt^{ma/ma}* sample.

AD case collections

The following collections of cases were used:

(1) **English adult severe AD** is early onset, persistent and severe disease, diagnosed by experienced dermatologists in secondary and tertiary care.

(2) **UK mild-moderate pediatric AD** includes cases collected from an English population birth cohort (n=177) and a Scottish Primary Care collection (n=161). AD is defined using the UK refinement of the Hanifin and Rajka diagnostic criteria.

(3) **Irish pediatric AD** cases were collected in secondary and tertiary care clinics in Ireland, defined by experienced dermatologists.

(4) **German AD** cases are adults and children diagnosed by experienced dermatologists and pediatricians using standard criteria for AD, in tertiary care clinics.

(5) **Scottish asthma cases with AD** are individuals with physician-diagnosed asthma in primary and secondary care, with parent- or self-reported history of ever having had AD.

The following matched control populations were used:

(1) **1958 Birth Cohort** comprises unselected English population controls.

(2) **English pediatric controls** were ascertained not to have AD at the age of 7-9 years. They are the hyper-normal controls from the same English population birth cohort from which the English mild-moderate pediatric AD cases were recruited.

(3) **Irish population controls** are healthy adult blood donors in Dublin, Ireland.

(4) **German population controls** include pediatric and adult cohorts from Germany.

(5) **Scottish population controls** are healthy adult blood donors from throughout Scotland.

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Table E1a
Primers for mouse SNP detection and positional candidate gene sequencing

Gene/mRNA	Location on mouse Chr3		F primer (5'-3')	R primer (5'-3')
4930564K09rik	82680531:82747696	exon 1	GAAAGGGACTTGGAGTGG	TAGTGTATTAGATGTGGCTCAG
		exon 2	CATAGAACAGGATAGTAGCACAG	CAGTGCAAAGCTGTGGTT
		exon 3	GATGCATGCATACTCCTAAGG	GCATCTTCCACTTCAAGGG
			GCAGACCTATTGCCGTTT	GATCCTAGTTCCTTCTCCTCAC
			CAGATATACATGGGATTTAAGG	TGGATGAGACTGCCATTC
			CAGCTATACTATATGTCCTGAA	GGATGATGAGGGCATAGAA
			CTGTGACTCTAGTTCCAAGGG	CAGTCAGCAGGTTGTTAAGG
			GCTACCTGGAACACAATGA	TAGGACCATATCCATTAGAC
4933430H15rik	87540562:87552864	exon 1	GCGAAATGGTAAAGATGGG	GGATTTTTTGGCTCGTCC
		exon 2-3	GCAAAGCAGCTTCCACTTG	AGGCCAAAAGTCATCTCAGG
		exon 4-5	CTCACGGGCTCACAATTCA	TCCCTGTGATTCTCTACAGAGA
		exon 6-7	CTATCCTTAGGCTCAGAT	AGAACAGAGTGAAGTTAAG
		exon 8	GCTTACAAGTCACTCTGTTCT	CAGCAGATTTTGTGAACATA
		exon 9	CAAAGTGAGCCATTTCCCTGT	CCAGAGCCCAGCTTCTCTT
		exon 10	CATTATAGCTGTCCCATGT	AGGAAGACCTCCTAAGGAAG
		exon 11	GTTTCTCCAGCACCTGGGAC	CATCTGACATCACTGGCAGCC
		exon 12	GGGATGGACTTGATTCTCAA	GTTTTAGGGCTGTTGAACGG
		exon 13	GTTTGTTTCCTGCAATTAG	CAAACACATGGAGATAGC
			GGAACCCAGCCTTCTCTGA	CCGGTGTGTAGACCTTCTCAG
			CTGGGCCAGAGCCAGTTGT	GGGAGGTCTCCAGATTCCA
			GACACTGAGAAGGCCTAGG	TCAAATGTAGCCAGCGTC
AA008399	87703360:87703930		CCCACATCAACCATCAAG	CAAAACCCAGGTCTTATG
AW764307	88516367:88517054		GCACCTTTAGGCACACTTG	TGTTGGCAGGGTTTATG
BB160298	87653139:87653787		TTACCCACTCTCTCCAAGG	TTTTGAAAGTGGAGGTTCC
BY722912	93075872:93076483		TGACATTGGAGAAATAGAGGTG	CCATCATTCTTGCCATTAGTAG
CN720784	87830051:87831030		AGTTCCAGGATAGCCAATG	TACCGCAAAGTGCCTAAG
			GGACTCCATTACACAAGC	TGACTTAGTGTGCTGCGTG
<i>Crnn</i>	92948710:92953707	intron 1	ATGGAGGAAGAGGCTATGC	ACAAAGGAAGACGGAAGC
D930015E06rik	83730775:83844469	exon 1	GCTGGGCTACGATTCATTC	GCGAACCCTCACAGATTTC
		exon 2	GGCTGGGCATTGTATTTG	TTAGAGACAGCCGTGGAGAC
		exon 3	CCAGAGGGTAGAGTTACAGGTG	TCTGAACAAGAGGGAAGGC
		exon 4	CTATTGATGATGGAGACTTTGG	CCAAACTTAGGGCTTCTCTG
		exon 5	GAGAAGCCCTAAGTTTGGTC	AAAGGGCAGGAAATCTCTC
		exon 6	GGTCCACATCAGAAGAGAAG	TGAGCCCATAGCCCAATAG
		exon 7-8	GCTGTTTGGAACTCACTGTATG	TTATGGATGAGGCAGGACC
		exon 9-10	TGGAGAGTCTATGGCTTTTTG	CCTGAGTGAAGTCCCAAC
		exon 11	CCTCCCCAAACTCTTCTTTAC	AACTGGGGTTCAGGAGAAG
		exon 12	GGTATTTCCTGAGTTGTATTGG	GCCACAAGTGAAGTAAAGC
		exon 13	TTTAGTCATAGCGAGTGCG	GCACAGAGGAAGGAAACTG
		exon 14	CACTGATGATTGTCCAGAGG	TCTCTCACAAAAGCACAAGC
		exon 15	CTCATAGTTTGTGAAGTTTGGC	TGCTGGCTGAAGTGCTAAC
		exon 16	CATTCTCTCTCTCTGAC	GCACAGTGACAAAGAAGGTG
		exon 17	TGTAGGATGAGGTCAAGAGTTG	CACTAAAAAGGAATGCTAAGGG
		exon 18	TGACTCTACCCTCCATTGG	AAATGTGTGTGTGCTGTG
		exon 19	TGGCATTTTAGTCTGTGAGAG	GGGAAAGAGTCAAACAACCTC
		exon 20	AAGTGAAGTGATGAAACCTGC	CCATAAGGTCCTCTCTAAAGC
DY243455	87335657:87336293		CCCTCCACATTTGTTCTATTTC	CAAGGTTTCTCTTTTAGCCC
<i>Flg</i>	93080173:93080723	intron 1	CACTGAGATTCGGGCTATG	TTCTTAGACTCTTCCTTTGC
<i>Flg2</i>	93001960:93005015	intron 1	TCTGGAAGGACAACACAGG	ACCATGTCACCTCAACGAG
GH455320	88508416:88509079		AGTGCTGGGAAAGAGGGTAG	TCTGGGGAAGTCTGGGATAC
gm6570	87483615:87484251		GGGAGGAAGCAAAGAAGAG	AGAATCACCTGGGTCAATG
gm9790	85719448:85720030		AGTCAAACCATTTGGCTCTC	TCTAACTAAAGCAAGCACTTCC
map6.26	92559223:92559820		GGAGAGGAAAAGTATGCCTG	GATAAGCATTTGGCACTGG
<i>Rptn</i>	93195844:93201987	exon 1	CCAACCAGAGTAGACAGTAAGG	TTATCACGAGGAAGAGAACAAC

		exon 2	TTTGGAGACATCCTTCGG TGGAACACTTGGACCGAGAC	TTATGATGGCAGGCTTGG CTCTCTGTCTGACTGTTGCC
S100A10	93357462:93366990	exon 1	GTTAGTCTGTTGGCAGAGTG	AAGGAGAACAGAACAGCGG
		exon 2	CCAATACCTGAAGTGCTGTG	CACCTGCAGATTGAGAGTTCTT
		exon 3	CCTTATGCACGTGGCTGTCA	CCTGAGAGGAGAATGCTTTCCC
<i>Tchh</i>	93244475:93251622	exon 1	TGAAATGAAAACAGGGGG	TCATCAGTGGTAATGCCC
		intron 1	GGTCTTGATGGCATTGTTAG	GCTGGAGAGATGGTTTAGCC
		exon 2	TACAGATGGGGTTGTGAGCCAC	AGTCTCTGCTCTTCATCGC
<i>TMEM79</i> (<i>Matt</i>)	88132575:88183555	exon 1	TTTGGAGACACAGGCAAG	TCTCTACAGCCAAGGGTCA
		exon 2.1	TTCTCTCCCACAGTGTCT	AATGGCTCCTCCGACTTAG
		exon 2.2	CAGAGGCACCTACTAAGTCG	AGTGGCAGGTTTCAGGATAC
		exon 3	GGTTTCTCTTCATTCTGCTG	AAGGCAATGGATTCAACAC
		exon 4.1	GGTGTTTGGAGTAGTATCGGC	TTTTCTTCTCCTTGCGC
		exon 4.2	ACACTTCTCTCTTGGGCAG	GCTTGAAGGCAGTTAGTTTC
		exon 4.3	CAAGCCTCTCTCTGTCTTCC	TGCTCCAGTAAGTTGTCC

Table Ib
Primers for human *MATT* gene PCR and sequencing

Gene/mRNA	Location on human Chr1		F primer (5'-3')	R primer (5'-3')
<i>TMEM79</i>	156254070:156262234	exon 1	GCCTGCTGCTACCAAATCT	CTTTACTCTATGTGATGCTCG
		exon 2.1	TGGACGGCATTTGATGTC	TGTAGGTCAATAGGCACGAAG
		exon 2.2	GACGATGCCAACCTGCTG	AGGGTAGGGTCAGAAAAGGC
		exon 3	TTCCCTTCACTTGACCTG	GAAATCGGCTTCCATCTG
		exon 4.1	TACCAGCCATACCCACCAAC	ACGGCTGTCCACCTTTTTC
		exon 4.2	CAGGGAAAAAGGTGGACA	TAACATCAACCACCGCAC

Table E1c
Primers for semi-quantitative RT-PCR (mouse)

cDNA murine primers	Location	F primer (5' - 3')	R primer (5' - 3')
<i>TMEM79/Matt</i>	chr3:88,133,776-88,136,947	CATCTTCTTCCCCTGTCTG	CAAGAGTGGCAGGAAAGTC
<i>Krt14</i>	chr11:100,068,255-100,068,804	ACTCACTCGTCACTTGCTCA	ATCTTGCTCTTCAGGTCCTC

Table EII
SNPs detected by sequencing in human *MATT* gene

Chromosome position	mRNA position	dbSNP rs# cluster id	Function	Reference allele/SNP	Protein residue	Amino Acid Position
1:156253031	-	rs28372828	5' near gene	G/C	-	-
1:156254988	142	rs192228711	5' UTR	T/C	-	-
1:156255456	610	rs6684514	missense	G/A	Val>Met	147
1:156255747	-	-	intron	T/C	-	-
1:156255833	-	rs2842883	intron	G/C	-	-
1:156256000	-	rs3795728	intron	T/C	-	-
1:156261491	1,458	rs6679145	3' UTR	G/C	-	-

Table EIII**Case definitions and demographic data for AD case collections and population-matched controls**

Case or control collection	Number of individuals	Names of collections	Phenotype definition
English adult severe AD cases	505	?	AD defined by experienced dermatologists; early onset persistent and severe disease in patients of white European ancestry living in or near London or Newcastle-upon-Tyne; cases recruited from secondary and tertiary care
UK mild-moderate pediatric AD cases	338	NCCGP and GenCEP	AD defined using UK Diagnostic criteria*; mild and moderate disease defined by the Three Item Severity score**; pediatric cases recruited from an English population birth cohort (NCCGP n=177, aged 7 to 9 years) and a Scottish Primary Care case collection (GenCEP n=161, aged 0 to 16 years)
Irish pediatric AD	724	NCRC-ADC	AD defined by experienced dermatologists; cases aged 0 to 16 years, recruited from secondary and tertiary care
German AD cases	1543	German tertiary care cases	AD defined by experienced dermatologists and pediatricians according to standard criteria***; tertiary care pediatric and adult cases recruited from University Hospitals in Germany
Scottish asthma cases with AD	1135	BREATHE and PAGES	Pediatric and young adult physician-diagnosed asthma cases with parent- or self-reported history of ever having had AD
English 1958 Birth Cohort	1919	1958 Birth Cohort	English population birth cohort
Non-AD English controls	538	NCCGP	English population birth cohort for whom AD was excluded at the age of 7 to 9 years
Irish adult population controls	1905	Trinity Biobank Controls	Irish adult blood donors from Dublin
German population controls	2005	KORA, PopGen and ISAAC	Population based German pediatric (ISAAC) and adult (KORA, PopGen) cohorts
Scottish population Controls	4189	Generation Scotland (GS3D)	Adult blood donors from throughout Scotland

* Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. Br J Dermatol 1994;131: 406–16.

** Acta Derm Venereol. 1999 Sep;79(5):356-9. Scoring the severity of atopic dermatitis: three item severity score as a rough system for daily practice and as a pre-screening tool for studies. Wolkerstorfer A, de Waard van der Spek FB, Glazenburg EJ, Mulder PG, Oranje AP.

*** Rajka G. Essential Aspects of Atopic Dermatitis. Berlin: Springer-Verlag; 1989.

Table EIV**Case-control analyses to investigate the association of rs6684514, FLG null mutations and AD**

Case/control comparison	cases, n	controls, n	rs6684514 and AD		rs6684514, <i>FLG</i> null mutations and AD		
			OR (95% CI)	p-value	rs6684514 OR (95% CI)	<i>FLG</i> OR (95% CI)	p-value
English adult severe AD vs English population controls from the 1958 Birth Cohort	505	1919	0.791 (0.674 to 0.929)	0.0038	0.709 (0.580 to 0.868)	3.248 (2.436 to 4.331)	0.0008
UK mild-moderate pediatric AD vs English pediatric controls without AD	338	538	0.770 (0.622 to 0.953)	0.0153	0.791 (0.637 to 0.981)	1.751 (1.260 to 2.433)	0.0328
Irish pediatric AD vs Irish adult population controls	724	1905	1.025 (0.896 to 1.172)	0.1300	0.981 (0.850 to 1.132)	4.238 (3.512 to 5.162)	0.7905
German AD cases vs German population controls	1543	2005	0.912 (0.822 to 1.011)	0.0794	0.860 (0.761 to 0.972)	4.891 (3.891 to 6.146)	0.0161

Logistic regression analysis was performed in Stata[®] 12.0 (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

Figure E3

Bioinformatics analysis of Tmem79/matrin Protein

1. Summary

A preliminary sequence analysis of TMEM79 (NP_115699.1 | CAI14162.1) was performed using ANNOTATOR analysis framework (Schneider et al. 2010; Ooi et al. 2009). There seems to be evidence from computational predictions and the comparison to published structures to support the following findings:

1. TMEM79/matrin and its orthologs are distantly related to the MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) superfamily many of whose members are involved in inflammation pathways.
2. Although sequence identity is very low, a multiple sequence alignment of MPGES1 and TMEM79 together with other canonical members of the MAPEG family shows that a large percentage of the key residues involved in glutathione binding in MPGES1 are also present in TMEM79 (see section 3).
3. Since MAPEG family members catalyze glutathione-dependent transformations of lipophilic substrates harvested from the lipid bilayer (Martinez Molina et al. 2008), experimentally establishing Glutathione binding of TMEM79 could serve as a first step to validate the above predictions and decide on whether to continue with a search for a lipophilic substrate (see section 4).

2. General Sequence Analysis

A segment based strategy for functional analysis was used. Around 30 algorithms were applied to try to distinguish non-globular segments from globular domains. The non-globular segments are often regions of low-sequence complexity or compositional bias, but may carry out important structural functions or contain signals for posttranslational modification or localization. On the other hand, conserved globular domains with a fairly stable three-dimensional structure might carry out enzymatic function or serve as an interface in protein-protein interactions or have general scaffolding roles. The results can be seen in (a) and show that TMEM79/matrin has a long N-terminal region of low sequence complexity. No further analysis of this region was undertaken. The C-terminal half of the protein is dominated by five predicted trans-membrane segments. After this sequence motif and architecture analysis, orthologous sequences were gathered which were subjected to a similar analysis as above to look for emerging

patterns. Subsequently, the analysis was extended to individual segments to not only include orthologs but also remote homologs, which were gathered with an iterative procedure.

The ortholog collection algorithm was used to find a number of orthologs and subsequent analysis of these found an enrichment for PFAM domain hits (PF01124) against the MAPEG protein family

Further evidence for membership in the MAPEG family was uncovered:

1. Inspection of the HMMER result on TMEM79 finds a sub-significant hit (E-value: 0.029) against the MAPEG domain (PF01124)
2. PSI-BLAST finds members of the MAPEG family starting from round 2.
3. The locally installed version of HHPRED (Soding et al. 2005) finds a clear hit (E-value: $9.7E-11$) against the MAPEG domain (PF01124)

A number of caveats should be mentioned:

- All the well-studied MAPEG members only have four TM segments, while TMEM79 has five. The region of homology clearly only extends to the last four segments of TMEM79.
- All MAPEG members appear to only as long as the TM segments together. There are no long N-terminal unstructured regions, as found in TMEM79/matrin.
- All known structures of MAPEG members show the formation of a homotrimer.

3. Comparison of TMEM79 and MPGES1

There are a number of publications that provide detailed structural information as well as results of site-directed mutagenesis for MPGES1 (Microsomal prostaglandin E synthase 1) (Jegerschöld et al. 2008), LTC4S (Leukotriene C4 synthase) (Molina et al. 2007; Ago et al. 2007) and FLAP (5-Lipoxygenase-activating protein) (Ferguson et al. 2007) with the last one lacking enzymatic activity.

A multiple sequence alignment was constructed using locally installed MAFFT (Katoh et al. 2005) and taking as input the orthologs of TMEM79, MPGES1 and the seed sequences for the MAPEG domain in PFAM (PF01124). Since the seed only contained xenopus sequences for LTC4S and FLAP, the rat and human orthologs were added

respectively. A small number of MAPEG seed sequences with very long loop regions were removed.

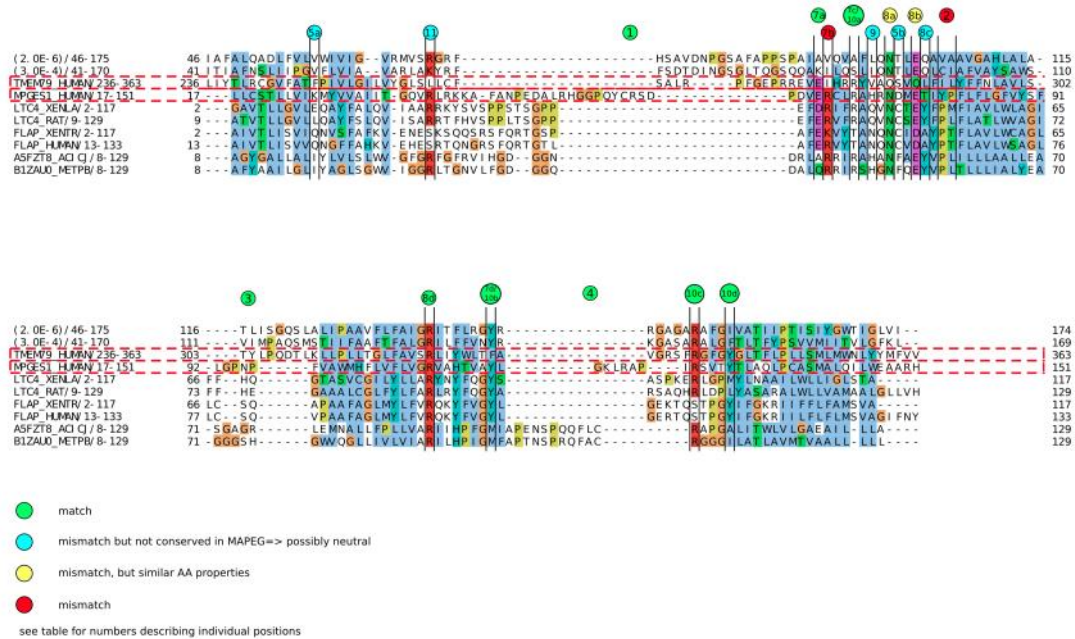
The alignment shown in (a), above, presents alignment with only the core members shown. The first two sequences are orthologs (YP_617596.1 and ZP_05250079.1) of TMEM79 which were retained to provide some visual guidance and context. The third and fourth sequence are TMEM97 and MPGES1 respectively (they are surrounded by a red dotted box). They are followed by two sequences of LTC4 and FLAP each. The last two sequences belong to the MAPEG seed and are again left there to provide some context.

The following tables (b-d), contain assessment which was done by comparing features and individual residues mentioned in (Molina et al. 2007) among the different sequences. Mismatches between residues are classified and color-coded as explained below. The same numbers and color-codes are indicated in the multiple sequence alignment shown in (a).

4. Summary

In summary, there seems to be a sufficient match between key residues TMEM79 of glutathione binding to merit further experimental investigation as a MAPEG-like protein.

(a) Multiple Sequence Alignment of TMEM79, MPGES1, LTC4 and FLAP and additional members of the MAPEG family



Key residues flagged here are detailed in b-d, below.

(b) Evidence for possible glutathione binding

No	MPGES1	TMEM79	LTC4S	FLAP	Assessment	Comment
10a	Arg-70	Arg	Arg	Thr		The two argenine/tyrosine pairs Arg-70/Tyr-117 and Arg-126/Tyr-130 are in close contact to the glycine and cysteine moieties of GSH
10b	Tyr-117	Phe	Tyr	Tyr		see 10a + 7d (Y117F is fully functional)
10c	Arg-126	Arg	Arg	Ser		stabilizes thiol group of GSH (also see 10a)
10d	Tyr-130	Tyr	Met/Leu	Tyr		see 10a
11	Arg-38	Leu	Arg	Ser		Arg-38 makes salt-bridge to carboxylate at end of GSH (other end with Arg-70 see 10a)

(c) Overall structure

No	MPGES1	TMEM79	LTC4S	FLAP	Assessment	Comment
1	✓	✓	✓	✓		TM1 followed by relatively large cytoplasmic loop region (highly variable)
2	Pro-81	-	Pro (shifted)	Pro (shifted)		conserved in MAPEG (with shift) Proline gives rise to a slight kink.
3	✓	✓	✓	✓		short loop on luminal side connecting TM2 and TM3
4	✓	✓	✓	✓		TM3 and TM4 connected by 4-residue loop
5a/b	Ion-pair (Lys-26/Asp-75)	Phe...	Glu Leu	Gln		Bending of TM1 through ion-pair (Lys-26/Asp-75) - not conserved
6	Arg-110	Arg	Arg	Arg		Arg-110 not exposed to lipid bilayer but rather involved in intermolecular contacts.

(d) Results of mutagenesis experiments on known MAPEG members

No	MPGES1	TMEM79	LTC4S	FLAP	Assessment	Comment
7a	Glu-66	Glu	Asp	Glu		stabilization of cytoplasmic face of TM2 towards TM3 by polar side chain residues (crucial for GSH binding)
7b	Arg-67	Ile	Arg	Arg		see 7a (mutagenesis indicates loss of function for R67A, but MGST3 has Cys and there are several Ala in the MAPEG seeds)
7c	Arg-70	Arg	Arg	Thr		see 7a (FLAP non-catalytic and no Arg!)
7d	Tyr-117	Phe	Tyr	Tyr		see 7a (Y117F mutant is fully functional)
8a	Asn-74	Gln	Asn	Asn		Further stabilization toward center through side-chain interactions (Asn-74, Glu-77, Thr-78 with Arg-110, His-113). Mismatch, but also polar (there are a few sequences in the MAPEG seed that have a Gln) and Ago et al. 2007 suggest that mutation of that position in LTC4S didn't affect activity
8b	Glu-77	Gln	Asp	Asp		see 8a. Dubious since Gln is not negatively charged and E77A shows no activity, but there are 2 sequences in the MAPEG seed that have a Gln at this position.
8c	Thr-78	Leu	Tyr	Ala		see 8a
8d	Arg-110	Arg	Arg	Arg		see 8a (conserved in all MAPEG)
8e	His-113	Tyr	Tyr	Tyr		see 8a
9	His-72	Val	Gln	Asn		His-72 forms salt bridge with Glu-77 (H72A mutant shows some activity)

match	mismatch but not conserved in MAPEG=> possibly neutral	mismatch, but similar AA properties	mismatch
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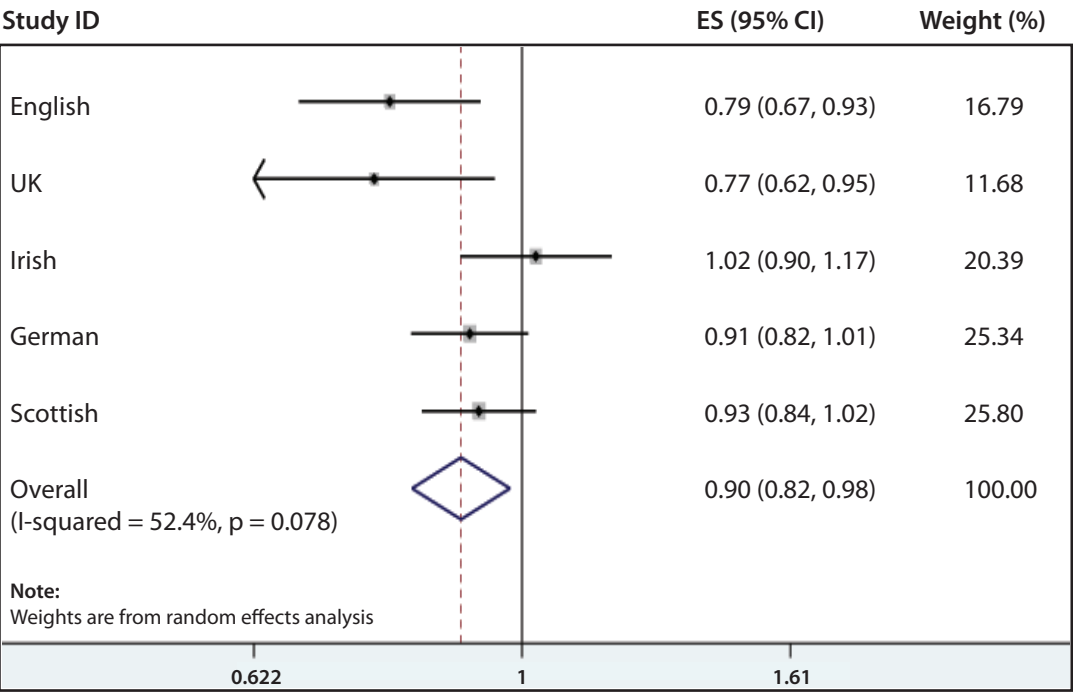
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Supplementary Figure 8



ONLINE FIGURE LEGENDS

Figure E1 *Breeding strategy for generating homozygous $Matt^{ma/ma}$ and $Flg^{ft/ft}$ mice.*

Figure E2 *Random recombination in chromosomal regions around the ma mutation with backcrossing, and CviQI restriction digest ma genotyping assay.*

(a) Backcrossing and intercrossing strategies employed to generate congenic $Matt^{ma/ma}$ mice on a C57BL6/J background, resulted in random recombination each generation, gradually replacing the chromosomal regions around the ma causative mutation with C57BL/6J genome, resulting in the homozygous critical interval carrying the mutation to be flanked by two uneven heterozygous genomic regions. (b) A genotyping assay was developed using CviQ I restriction digestion. The 644 bp PCR fragment derived from WT animals contains two CviQ I sites, one of which is knocked out by the mutation. In this restriction fragment length polymorphism assay, WT mice yield fragments of 380, 186 and 78 bp; bands of 458 and 186 bp are present in congenic $Matt^{ma/ma}$, JAX $Matt^{ma/ma}$, and DM mice.

Figure E3 *Mattrin sequence homology.*

Bioinformatics analysis of the weak sequence homology between mattrin and members of the MAPEG family of proteins.

Figure E4 *Immunofluorescent staining for mattrin in human biopsies, and TMEM79/MATT expression in human tissue.*

Immunofluorescence staining of human epidermis from scalp (a) and cheek (b), shows strong expression of mattrin (green) within the granular layer of the epidermis. In (c) longitudinal and (d) transverse cryosections of human hair follicles, immunofluorescence staining revealed that mattrin is located in the inner root sheath, internal to outer root sheath marker keratin K17 (red). (e) Screening a cDNA panel of 48 human tissues by QRT-PCR revealed that *TMEM79/MATT* is highly expressed in the skin, prostate and cervix, all of which are tissues with a high component of stratified squamous epithelium.

Figure E5 *Matt^{ma} mice develop AD-like inflammation, elevated TEWL and atopy.*

(a) Macroscopic clinical scoring. Data represents the mean; error bars represent $\pm$ SEM, from 25-30 mice per strain, scored longitudinally. (b) DM, *Matt^{ma/ma}* and *Flg^{ft/ft}* mice have elevated dermal cell infiltration, acanthosis and hyperkeratosis, relative to WT mice, with DM and *Matt^{ma/ma}* mice having increased dermal cell infiltration, acanthosis and hyperkeratosis relative to *Flg^{ft/ft}* mice. Cell numbers were counted, and acanthosis and hyperkeratosis scored, on 15-20 HPF (x1,000) on hematoxylin and eosin-stained sections from WT, DM, *Matt^{ma/ma}* and *Flg^{ft/ft}* mice. (c) Elevated TEWL readings in DM and *Matt^{ma/ma}* mice in comparison to WT and *Flg^{ft/ft}* mice. (d) Total-IgE in the serum is elevated in DM, *Matt^{ma/ma}* and *Flg^{ft/ft}* mice, relative to WT controls. Data represent the mean and error bars represent $\pm$ SEM (a,b,c) or min and max (c,d). Student's t-test or two-way ANOVA was used to determine statistical differences between groups. NS- $P > 0.05$ * $P < 0.05$, *** $P < 0.001$.

Figure E6 *Individual clinical score parameters, and severe AD pathology in Matt^{ma} mice.*

(a) Clinical scoring assessed by the parameters of pruritus, erythema, edema, erosion and scaling. Data represent the mean; error bars represent $\pm$ SEM, from 25-30 mice scored longitudinally. (b) Severe pathology is evident in individual DM and *Matt*^{ma/ma} mice by 32 weeks, with some mice exhibiting profound lesions and excoriation, particularly on the neck and ventral flank, with occasional incidence of blepharitis and ocular edema.

Figure E7 *Hair fiber and bulb morphology.*

(a) Scanning electron microscopy analysis of hair fibers from *Matt*^{ma/ma} and DM mice shows defective cuticular morphology, with fragile hair fibers prone to splitting and breakage. In contrast, hair fibers from WT and *Flg*^{fl/fl} mice show regular cuticle morphology. Scale bar, 20 μ m. (b) Representative photomicrographs shows that the hair follicle bulbs (arrows) are at different depths within the subcutis of *Matt*^{ma/ma} mice at 9-days during morphogenesis, compared to heterozygous *Matt*^{ma/+} littermates, leading to hair follicle misorientation. Scale bar, 100 μ m.

Figure E8 *Forrest plot showing results of random effects meta-analysis of five case-control studies to investigate the association of rs6684514 and AD.*

Study populations used were: English, English adult severe AD vs English population controls from the 1958 Birth Cohort; UK; UK, UK mild-moderate pediatric AD vs English pediatric controls without AD; Irish, Irish pediatric AD vs Irish adult population controls; German, German AD cases vs German population controls; Scottish; Scottish asthma cases with AD vs Scottish population controls. ES = estimated odds ratio; CI = confidence interval. Meta-analysis carried out using the “metan” function in Stata® (StataCorp, College Station, Texas).

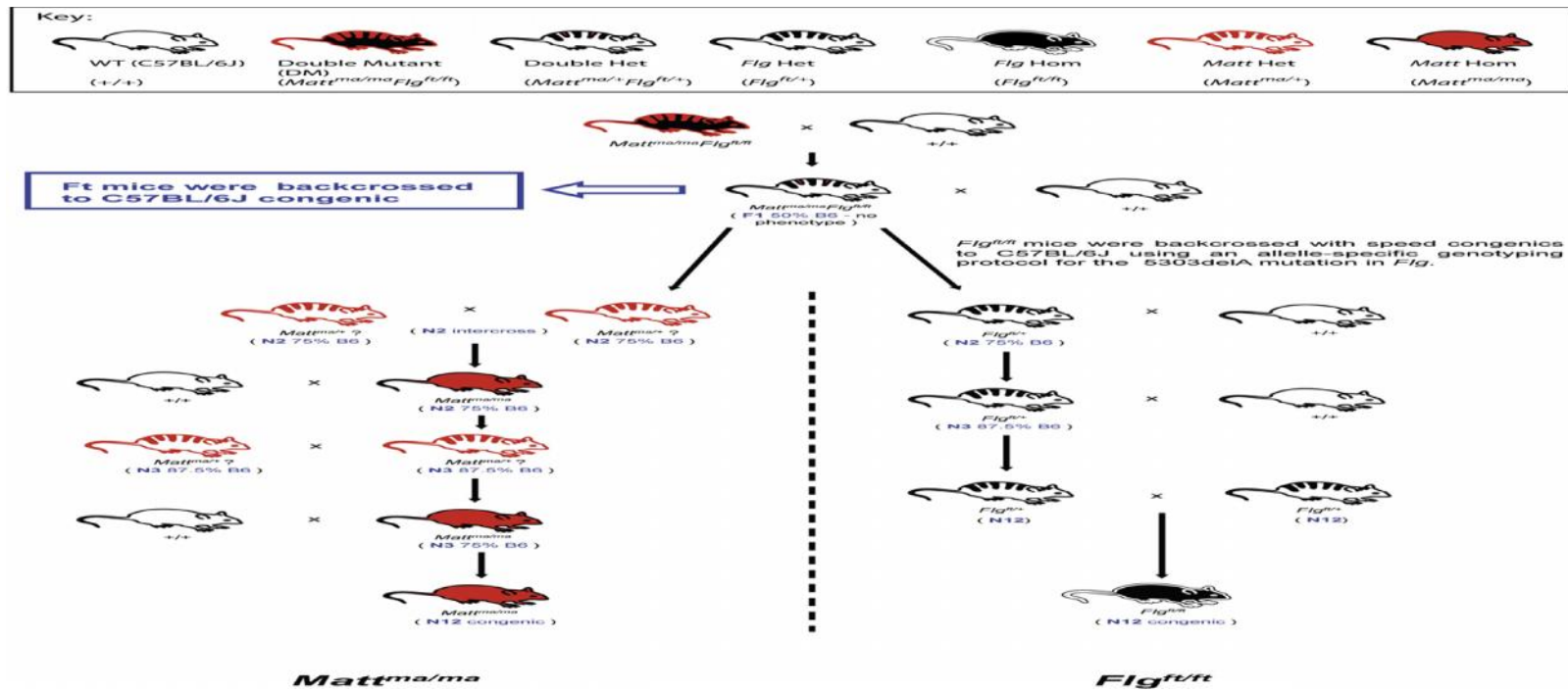


Fig E1

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***Tmem79/Matt* is the matted mouse gene and is a predisposing gene for atopic dermatitis in human subjects**

Journal of Allergy and Clinical Immunology null 2013 null

<http://dx.doi.org/10.1016/j.jaci.2013.08.046>

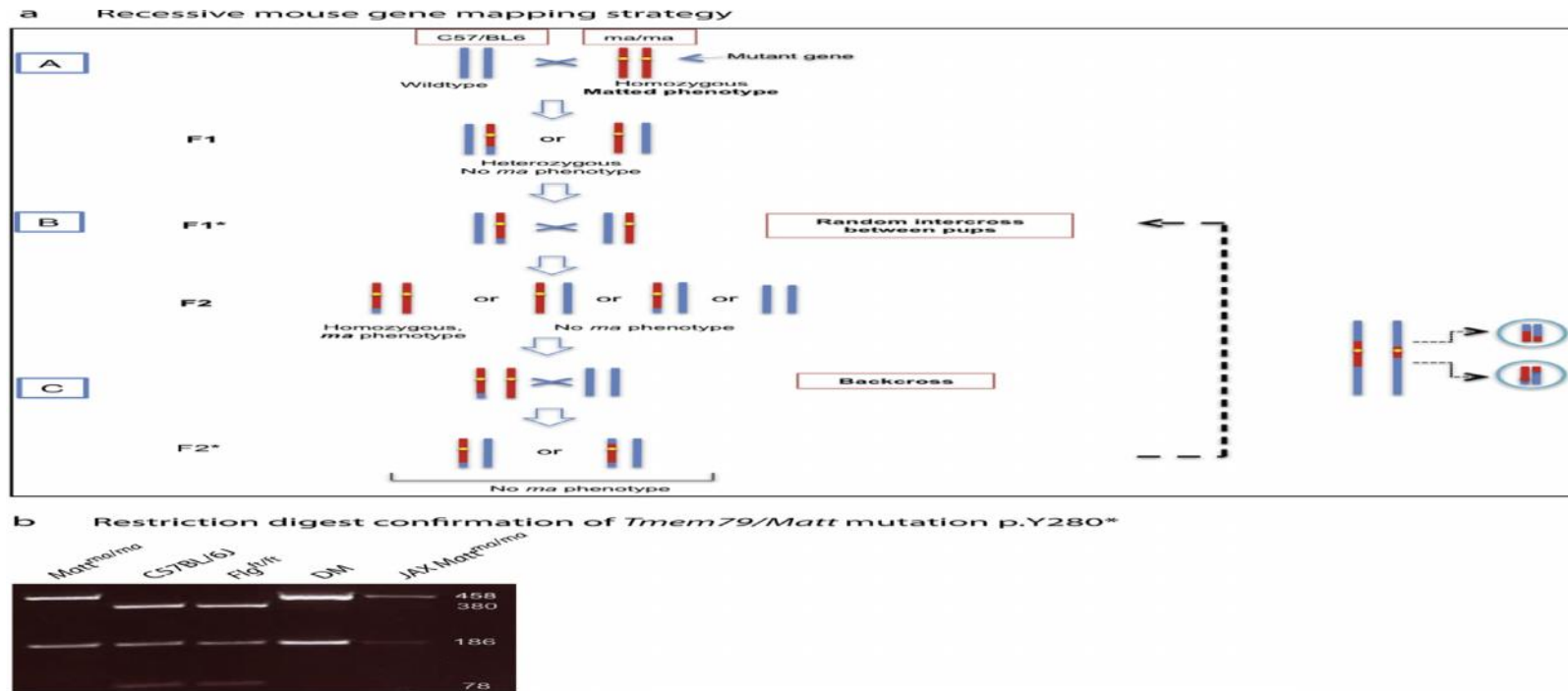


Fig E2

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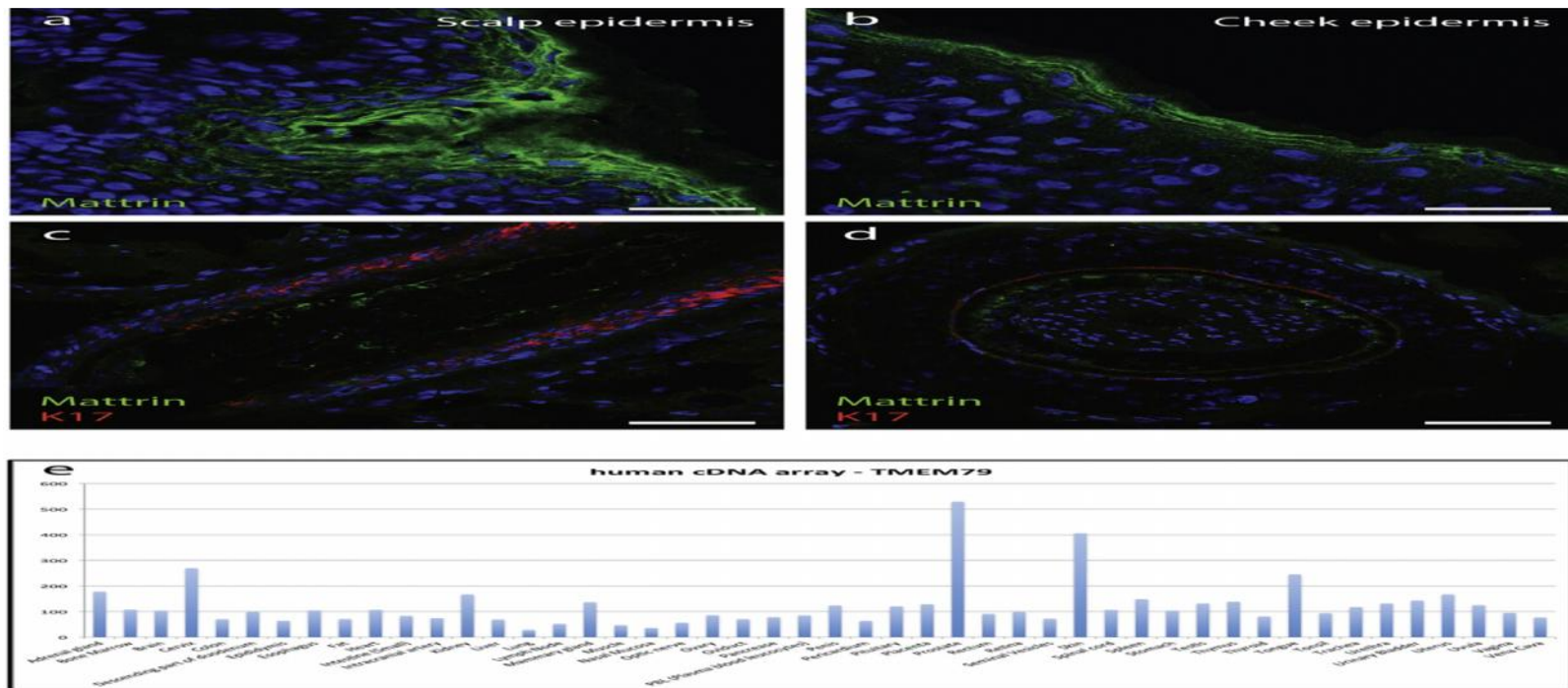


Fig E4

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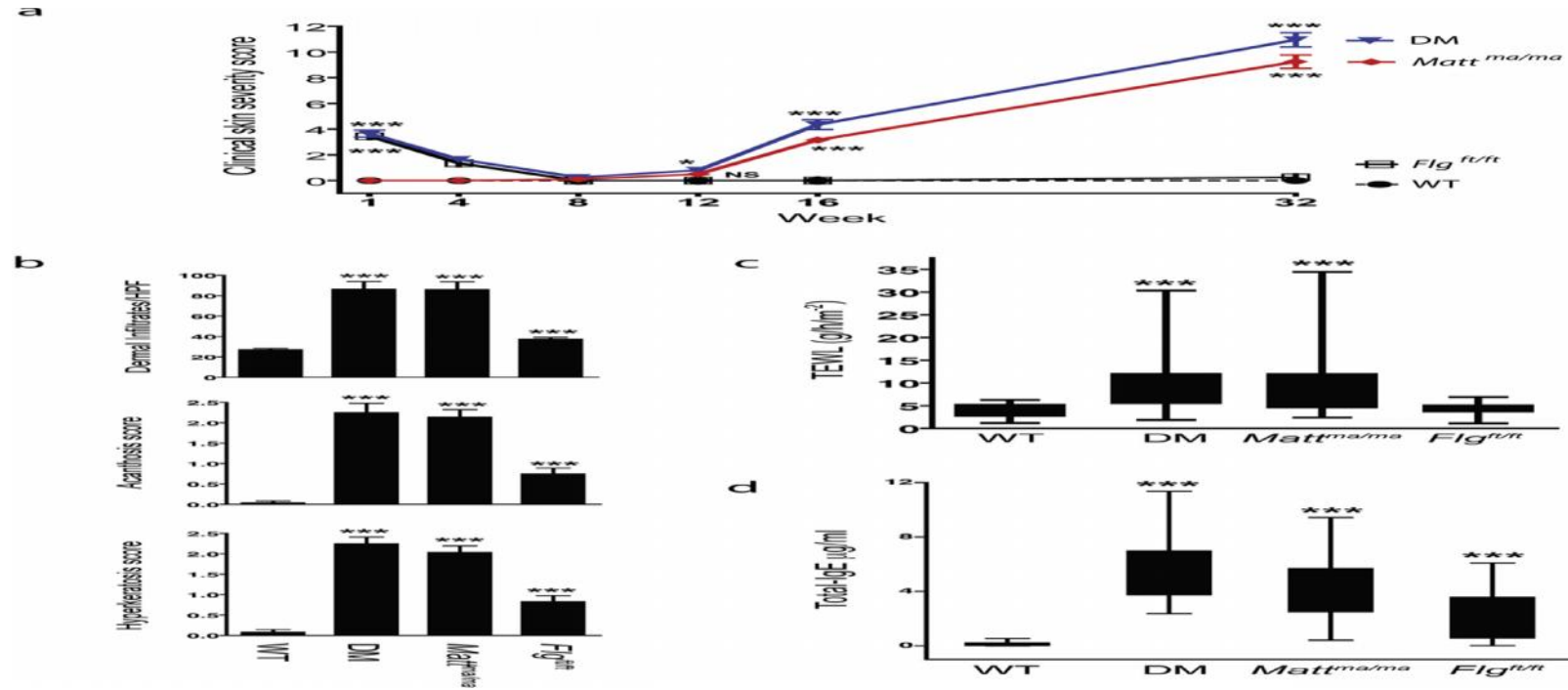


Fig E5

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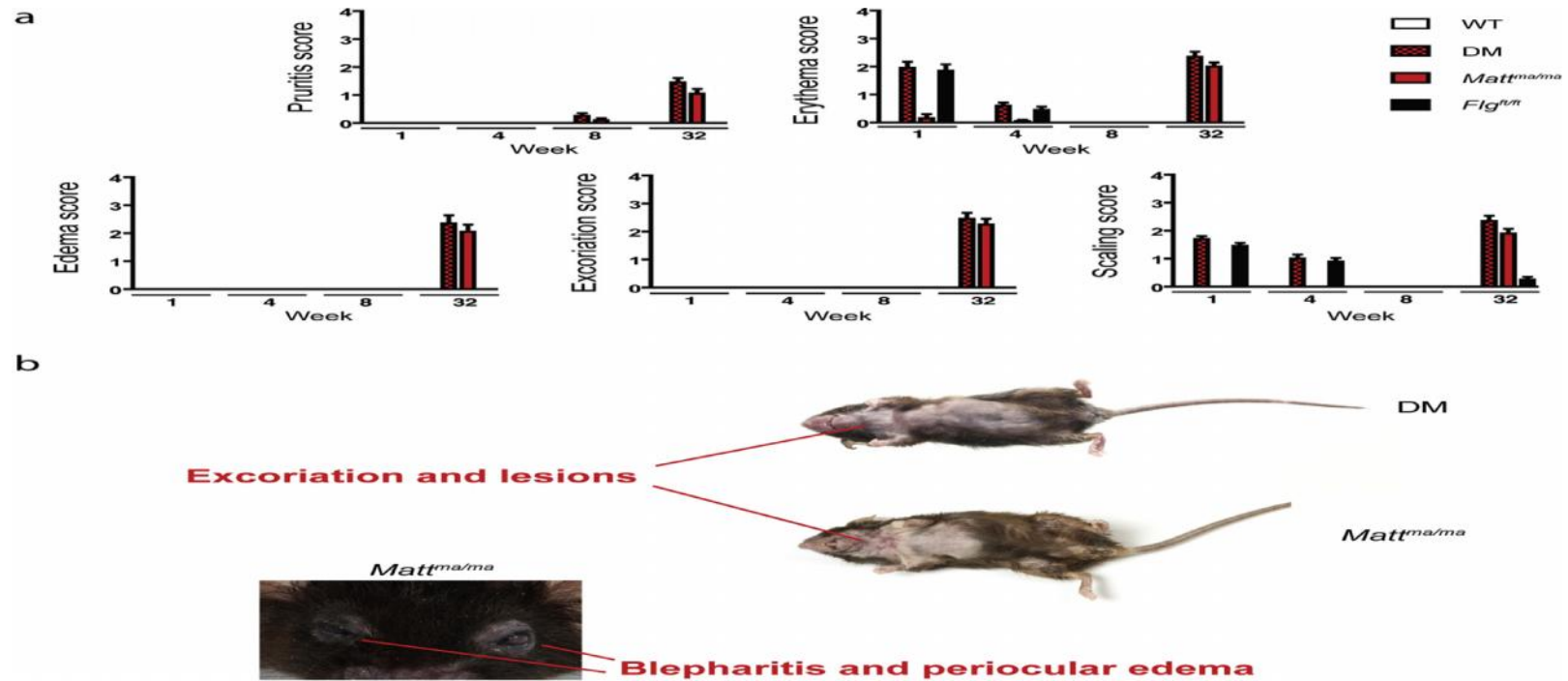


Fig E6

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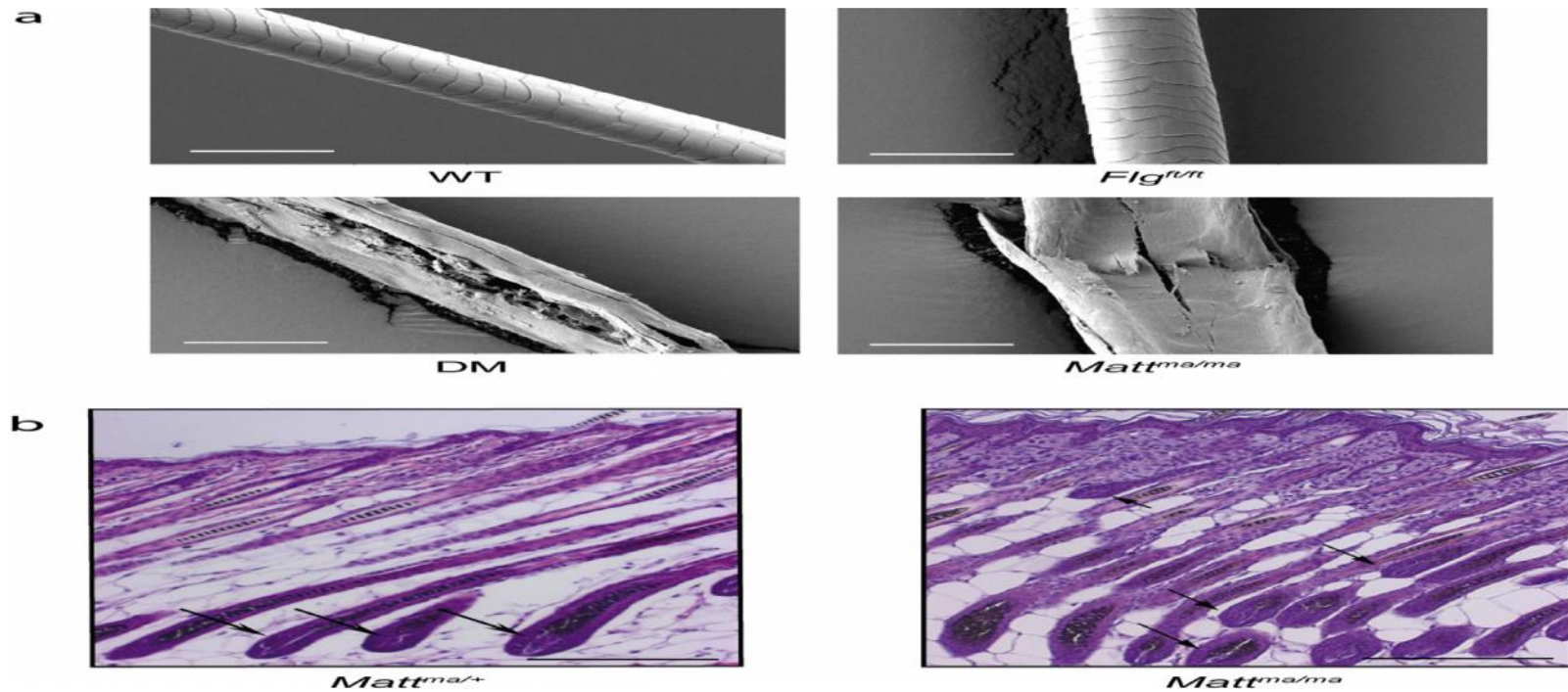


Fig E7

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