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

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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.

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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 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.

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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 loci[4-7]; however, the major and only functionally characterized genetic factor is the filaggrin gene *(FLG)*, which encodes the skin barrier protein filaggrin.[3] 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.