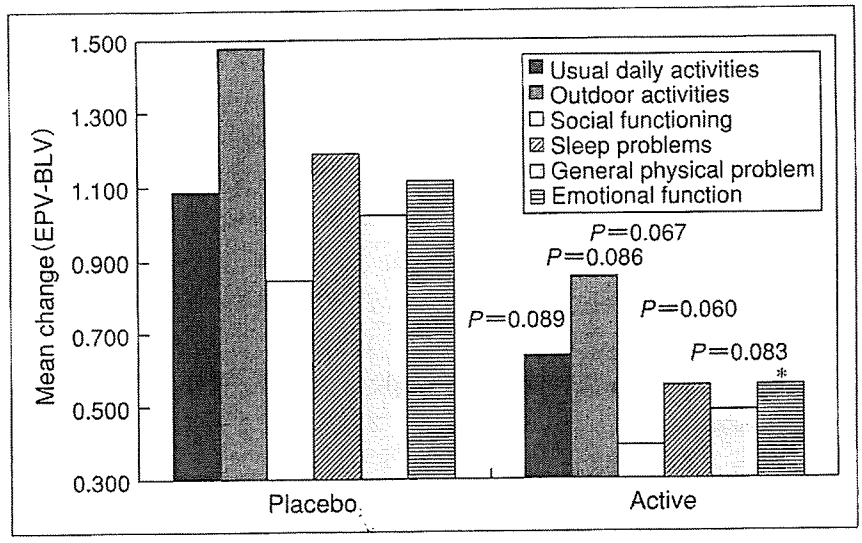


図6　QOLスコアの変化(全国6施設)

は少なく[16][17]，スギに対しても舌下免疫療法が有効かどうか検討した．今回の検討では舌下免疫療法は，スギ花粉飛散後期の症状増悪を抑制し，全般的にはシーズンを通して重症度を軽症化させる傾向にあった．抗原特異的に治療効果を発生させる免疫療法は，抗原曝露による鼻粘膜過敏性の亢進を抑制することによって，シーズン後半の症状の増悪を制御しているのかもしれない．花粉シーズン中のQOLの悪化についても，舌下免疫療法は最小限に抑えることができた．さらに全身性の副作用もまったく発現せず，安全に治療を継続できた．

今後もより多くの患者について詳細な検討を行う必要があるが，スギ花粉症に対する舌下免疫療法は海外の他のアレルゲンの報告と同様に安全性が高く，治療効果も期待できる方法であると考えられる．

文　献

1) 奥田　稔．スギ花粉症免疫療法の長期予後．アレルギー 2006；55：655.
2) Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double-blind study. Allergol Immunopathol (Madr) 1990；18：277.
3) Mungan D, Misirligil Z, Gürbüz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma : a placebo controlled study. Ann Allergy Asthma Immunol 1999；82：485.
4) Guez S, Vatrinet C, Fadel R, et al. House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis : a double-blind, placebo-controlled study. Allergy 2000；55：369.
5) Di Rienzo V, Puccinelli P, Frati F, et al. Grass pollen specific sublingual/swallow immunotherapy in children : open-controlled comparison among different treatment protocols. Allergol Immunopathol (Madr) 1999；27：145.
6) Hordijk GJ, Antvelink JB, Luwema RA. Sublingual immunotherapy with a standardised grass pollen extract；a double-blind placebo controlled study. Allergol Immunopathol (Madr) 1998；26：234.
7) Clavel R, Bousquet J, André C. Clinical efficacy of sublingual-swallow immunotherapy : a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. Allergy 1998；53：493.
8) Pradalier A, Basset D, Claudel A, et al. Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. Allergy 1999；54：819.
9) Valle C, Bazzi S, Berra D, et al. Effects of sublingual immunotherapy in patients sensitised to *Ambrosia*. An open controlled study. Allergol Immunopathol

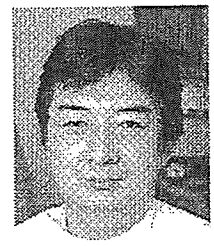
(Madr) 2000 ; 28 : 311.
10) Troise C, Voltolini S, Canessa A, et al. Sublingual immunotherapy in *Parietaria* pollen-induced rhinitis : a double-blind study. J Investig Allergol Clin Immunol 1995 ; 5 : 25.
11) Purello D'Ambrosio F, Gangemi S, Isola S, et al. Sublingual immunotherapy : a double-blind, placebo controlled trial with *Parietaria judaica* extract standardized in mass units in patients with rhinoconjunctivitis, asthma, or both. Allergy 1999 ; 54 : 968.
12) 鼻アレルギー診療ガイドライン作成委員会．鼻アレルギー診療ガイドライン．2005年版(改訂第5版)．東京：ライフ・サイエンス；2005.
13) Okubo K, Gotoh M, Fujieda S, et al. A randomized double-blind comparative study of sublingual immunotherapy for cedar pollinosis. Allergol Int 2008 ; 57 : 265.
14) Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. Ann Allergy Asthma Immunol 2003 ; 91 : 288.
15) 大久保公裕，小林　慎．花粉症治療における労働生産性の意義．Allergology & Immunology 2007 ; 14 : 218.
16) 大久保公裕，後藤　穣，島田健一，ほか．スギ花粉症に対する舌下抗原特異的免疫療法―パイロットスタディー―．日鼻誌 2002；41：30.
17) Gotoh M, Okubo K. Sublingual immunotherapy for Japanese cedar pollinosis. Allergol Int 2005 ; 54 : 167.

*　　*　　*

1 スギ花粉抗原とヒノキ花粉抗原の関連性

The relation between Japanese cedar allergen and Japanese cypress allergen

日本医科大学千葉北総病院耳鼻咽喉科
後藤　穣（ごとう　みのる）



後藤　穣
1991年日本医科大学医学部卒業。93年静岡済生会総合病院耳鼻咽喉科派遣，94年日本医科大学付属病院耳鼻咽喉科助手，96年日本耳鼻咽喉科学会認定専門医，2002年日本アレルギー学会認定専門医，04年日本医科大学耳鼻咽喉科学講師，日本アレルギー学会代議員。
研究テーマは鼻アレルギー，花粉症。

Key words：スギ花粉，ヒノキ花粉，共通抗原性

Abstract

スギ花粉とヒノキ科花粉では主要抗原のアミノ酸配列が80％程度共通しており，分子構造的に類似した抗原と考えられる。これまでは，スギ花粉症といえば，ヒノキ花粉症については副次的なものとして捉えられてきたが，ヒノキ花粉の増加による症状の遷延化や植林面積の広さを考えると，今後ヒノキ花粉症がクローズアップされる可能性がある。花粉数が予測どおり増加し，4月，5月に重症の患者が増えればヒノキ花粉症に対する対策を考慮する必要が出てくるだろう。

はじめに

スギ花粉症は奥田の疫学調査によれば約13％の有病率がある疾患で[1]，近年の増加スピードを考えると国民病といっても過言ではない。従来はスギ花粉症と言えば，言外にはヒノキ花粉症も含まれることが多かった。その理由のひとつとして，スギ花粉とヒノキ科花粉では主要抗原のアミノ酸配列が80％程度共通しており，スギ花粉症の治療がそのままヒノキ花粉症の治療としても十分通用すると考えられてきたからである。

しかし，近年のヒノキ花粉の増加による症状の遷延化や植林面積の広さを考えると，今後ますますヒノキ花粉症はクローズアップされる可能性があり，スギ花粉症とヒノキ花粉症をそれぞれ独立した花粉症として考える必要が出てくるかもしれない。実際にスギ花粉症に対して抗原特異的減感作療法（免疫療法）を施行し，3月中までは症状を良くコントロール出来ていても4月から5月にかけては重症になり，免疫療法の効果が少ないと感じられる症例を経験することが少なくない。このような臨床的な事実から最近になりヒノキ花粉症に対して注目が集まってきている。

1. スギ・ヒノキ花粉の特徴

花粉症の原因になる花粉は風媒花の植物の花粉であり，草本と木本に大別される。草本

にはgrassとweedがあるが，grassはカモガヤやオオアワガエリなどのイネ科花粉があり，weedにはブタクサ，ヨモギなどのキク科の植物がある。木本にはスギ科，ヒノキ科の他に，カバノキ科が重要である。

スギ花粉は直径30～40 μ mで表面に小突起（オービクルまたはパピラ）がある。主要抗原としてCry j1とCry J 2が同定されている。1月初旬には飛散が観測されるが，飛散開始日は関東では2月中旬ごろで，3月下旬まで飛散が続く。

ヒノキ花粉はスギよりも小型で，直径30 μ m程度である。表面に突起はない。主要抗原としてCha o 1とCha o 2が同定されている。スギよりも遅れて3月中旬以降に飛散が始まり，5月上旬まで飛散する。形状的には両者に特徴があるものの，顕微鏡でプレパラート上のスギ花粉と正確に区別するには習熟が必要である。ヒノキ科花粉には，ヒノキ，アスナロ，サワラなどがあるが区別することは出来ない。したがって，ヒノキ花粉と表記せず，ヒノキ科花粉と表現するほうがより正確である。

2. スギ・ヒノキ花粉の共通抗原性

花粉アレルゲンは，一般的に植物の分類，すなわち科や属が近いほど共通抗原性を持つ頻度が高くなる。キク科のブタクサ属とヨモギ属では，ヨモギとブタクサの共通抗原性は低いが，ブタクサ属とヨモギ属の中の植物同士は共通抗原性が高い。

共通抗原性の程度は，分子レベルのアレルゲン蛋白の構造が影響している。グループ1とグループ2についてスギ花粉抗原とヒノキ花粉抗原の一次構造と比較すると，Cry j 1とCha o1では約80％，Cry j 2とCha o 2では約74％の類似性が見られる（図1，2）[2]。紀によれば，この抗原性の類似は，スギ，ヒノキだけでなく米国のMountain Cedar花粉にも当てはまる。しかし，スギ花粉症患者の血清を使用してRAST抑制試験を行うと，Cry j 1やCry j 2で抑制されてもCha o 1やCha o 2では抑制されないケースがあり，IgE抗体がCha o1, Cha o 2よりCry j 1, Cry j 2に強い親和性を持っている[3]。この事実は，臨床的にヒノキだけが感作している症例が存在しないということの裏づけになるだろう。

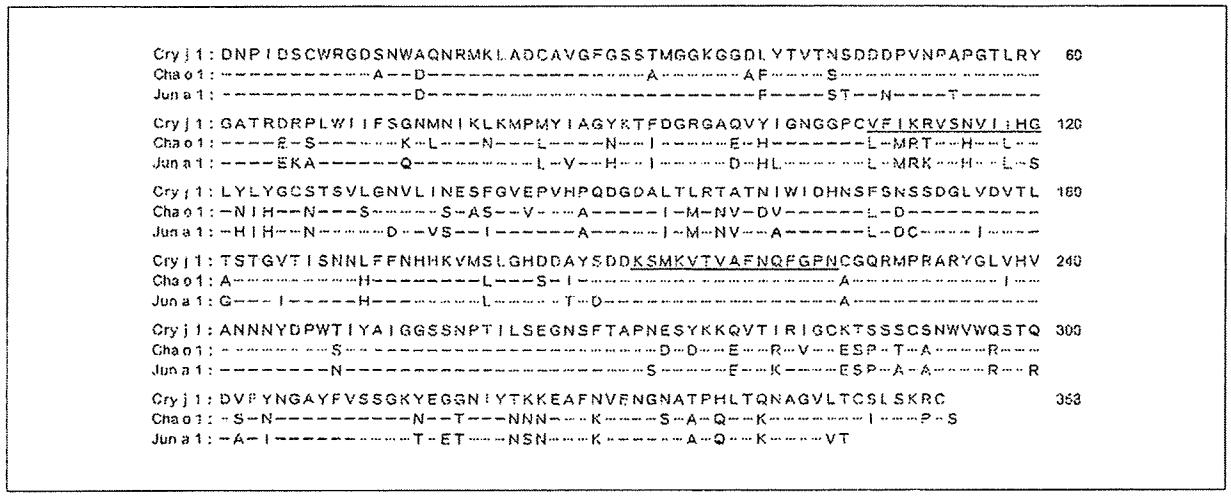


図1　スギ花粉抗原の一次構造（グループ1）（文献2より）

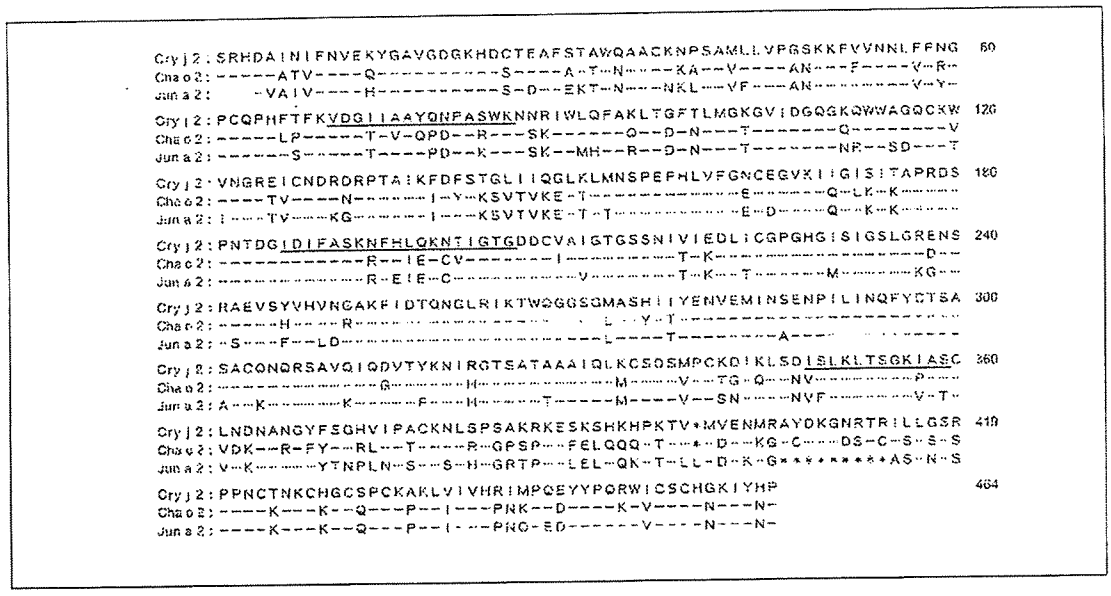


図2　スギ花粉抗原の一次構造（グループ2）（文献2より）

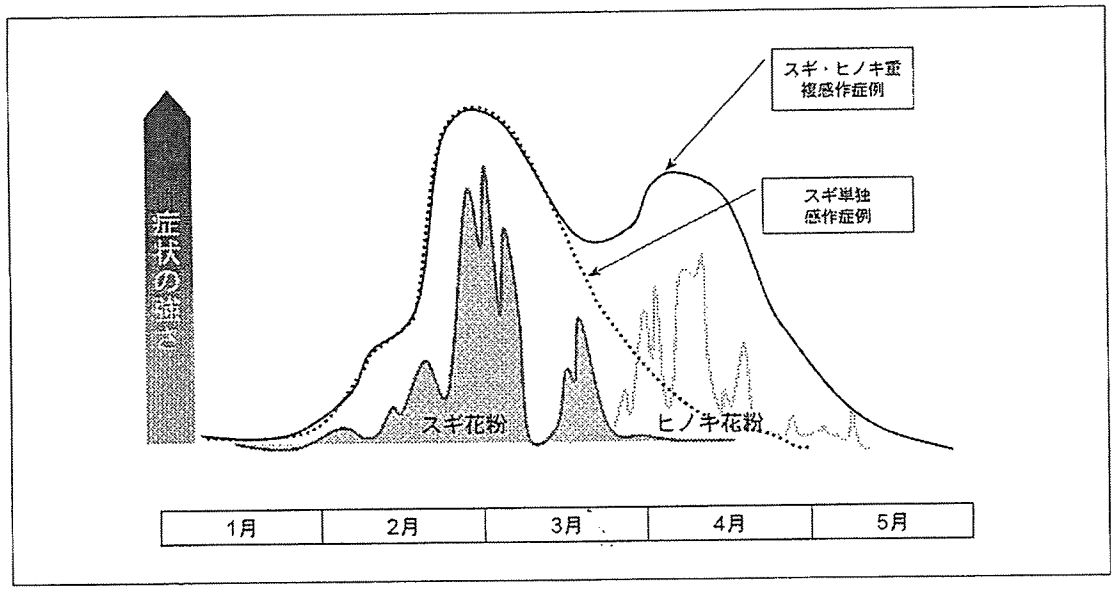


図3　スギ・ヒノキ花粉症患者の症状の変化

3. 臨床的な事実から

スギ花粉とヒノキ花粉は，共通抗原性があるため従来は一連の花粉症として捉えられることが多かった。季節的にほぼ同時期に花粉が飛散することや，スギ特異的IgE抗体陽性者のほとんどがヒノキ特異的IgE抗体陽性であることも関係している。実際に，スギ特異的IgE抗体だけが陽性のスギ花粉症患者も4月，5月まで症状が遷延化することも多いので，スギ，ヒノキの特異的IgE抗体がともに陽性の患者と臨床的には区別がつきにくい（図3）。

しかし，スギ花粉エキスで特異的免疫療法を行っている患者のなかには，スギ花粉飛散

時期には症状がなく，ヒノキ花粉が飛散し始めると花粉症症状が起きる例を経験する。湯田の報告でも，標準化スギ花粉エキスで治療していてもヒノキ花粉特異的IgE抗体陽性の症例ではシーズン後半の有効性の低下が示されている[4]。またスギ花粉症に対する舌下免疫療法の有効性判断にも，ヒノキ抗体の有無が影響する[5]。これらのことから，花粉同士に共通性があっても実は別々にアレルギー反応を引き起こしていると考えるのが自然である。

また，花粉曝露室を用いて人工的な花粉曝露実験を行うことが可能になった。花粉症は代表的なI型アレルギー疾患なので，抗原誘発によって明確に症状が出現する。抗原の量や曝露時間を変化させることによって，症状の推移や治療の有効性を検討できる。日本でもEEU(environmental exposure unit) Wakayamaを筆頭に大阪，東京，千葉にスギ花粉曝露室がつくられたが，東京都新宿区にあるOHIO chamberでは，スギ花粉だけでなくヒノキ花粉を使った曝露実験が可能である[6]。スギ・ヒノキ重複感作例を対象に検討すると，スギ花粉曝露によって誘発される症状とヒノキ花粉によって誘発される症状は同様であり，2種類の抗原に対して同じく反応することが示された。

終わりに

スギ花粉とヒノキ花粉は構造的に類似しており，スギ花粉症患者は多くがヒノキ花粉特異的抗体陽性である。これまでは，ヒノキ花粉の飛散量が少なかったため，スギ花粉症にカバーされヒノキ花粉症の症状が明確ではなかった。人工林の植林面積を見ると，ヒノキ林の占める面積は大きく，樹齢を考えると花粉を大量に産生する時期に入ってきている。近い将来には，スギ花粉症だけではなくヒノキ花粉症に対する対策，例えばヒノキ花粉症に対する治療用エキスの開発や花粉飛散情報の充実などが必要になる可能性が高い。

参考文献

1) Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. Ann Allergy Asthma Immunol. 2003 Sep;91(3):288-96.
2) 紀光助．スギ花粉症・ヒノキ花粉症－抗原解析からペプチド免疫療法に向けて－．感染・炎症・免疫．32巻2号 150-153, 2002
3) 安枝浩．花粉アレルゲンの交差反応性．花粉症研究会会報12号 2-9, 2001
4) 湯田厚司，服部玲子，坂井田寛，三品朋子，鈴木あゆ美，竹内万彦，臼井智子，間島雄一．スギ花粉症に対する免疫療法のヒノキ科花粉症への効果．日本鼻科学会会誌，46巻2号 109-113，2007.
5) 堀口茂俊，岡本美孝，米倉修二，大川徹，國井直樹，山本陛三郎，櫻井大樹，花澤豊行．スギ花粉エキスを用いた免疫治療へのヒノキ花粉感作の影響．アレルギー57巻5号 558-561，2008.
6) 橋口一弘，湯懐鵬，末松潔親，後藤穣，大久保公裕．OHIO Chamberでのヒノキ花粉曝露による症状発現の検討．アレルギー57巻3-4 423, 2008

リアルタイムモニター飛散数の情報のあり方の研究と舌下ペプチド・アジュバント療法の臨床研究
舌下免疫療法における網羅的蛋白解析と経年的効果の検討

研究分担者　藤枝重治　福井大学医学部耳鼻咽喉科・頭頸部外科教授
研究協力者　野口　恵美子　筑波大学人類遺伝学講師
牧野　友香　筑波大学人類遺伝学大学院生
湯田　厚司　三重大学医学部耳鼻咽喉科講師

研究要旨
これまでのプラセボ対照二重盲検試験の結果から、スギ花粉症に対して舌下免疫療法の有効性は実証できた。しかし具体的な投与スケジュールや投与期間などの検討は行われていない。そこで今回、オープン試験による舌下免疫療法施行期間による臨床症状の違い、自己評価の違いを検討した。また舌下免疫療法の効果発現機序を同定することも大切なことであるが、これまでまだわかっていない。そこで全蛋白の変化を調べることができる網羅的蛋白解析法を用いて、舌下免疫療法プラセボ対照二重盲検試験前後の血漿の検討を行った。その結果、舌下免疫療法継続2年目以上において、1年目に比較し有意に症状と薬物使用の改善を認めた。免疫治療前の花粉症症状を対照とした Visual analog score(VAS)では、1年目で52%、2年目で65%、3年目で70%、4年目で82%の症状軽減となり3年目と4年目が1年に比較して有意差を認めた。週1回投与と隔日投与の無作為割り付け試験では、有意差をつかなかったが、隔日投与の方 symptom-medication score、medication score、QOL score のいずれも低くなった。以上のことは舌下免疫療法の回数は多い方がよく、最低2年間のシーズンが必要なことが判明した。網羅的蛋白解析では5種類の蛋白を同定しえた。そのうち Apolipoprotein A IV（アポ A4）が臨床効果と有意に相関し、症状が改善した者に高い誘導が認められ、改善が乏しかったものでは低値を示していた。リコンビナントアポ A4 は、CryJ1 刺激によるヒト好塩基球のヒスタミン遊離率を抑制した。二重盲検試験において実薬群では、プラセボ群に対して、in vitro 末梢リンパ球での CryJ1 刺激によるヒスタミン遊離試験においても有意に低値を示した。これらのことはアポ A4 のアレルギー治療薬として、また臨床マーカーの可能性を示唆するものと考えている。

A. 研究目的

舌下免疫療法がスギ花粉症に効果があることは、これまでの4回の2重盲検試験において実証できたが、実際治療方法については、まだ十分に検討されていない。本年度は、何年間の舌下免疫療法が必要なのか、経年的に行うとどのように効果が変化するのか、抗ヒスタミン薬などの薬物併用はどのようになるのか調べることとした。

一方で、舌下免疫療法の効果発現機序を検討することも重要な課題である。その方法として、遺伝子多型、遺伝子発現、蛋白などいくつかの対象を一気に大量に検討できる網羅的解析がある。その中でも網羅的蛋白解析では、全蛋白の変化を検討することができ、予想もしえない遺伝子や蛋白が機能していることを見出すことができる画期的な方法である。本研究では、スギ花粉症に対する二重盲検試験の血漿を用いて、この網羅的蛋白解析を行い、臨床結果と相関し臨床効果発現の指標となる因子の同定を試みた。

B. 研究方法

平成17年度から平成20年にかけて実薬と知って舌下免疫療法を行った患者において、治療効果がどのように変化したかをオープン試験として検討した。治療効果は、花粉症日記（くしゃみ回数、擤鼻回数、鼻閉の程度:症状スコア）、QOL スコア、内服点鼻の使用内容（薬物スコア）にて判定した。

また投与回数を週1回と隔日投与の2群をつくり、37名無作為振り付け群間試験を行った。

舌下免疫療法前後で採血後、血漿を回収し、網羅的蛋白を行った。解析法は、Ettan DIGE システムにて二次元電気泳動を行い、Decyder により各スポットの発現強度を GeneSpring により解析した。統計的有意な変化を示しているスポットについて質量分析（mass spectrometry）を

行った。ゲルを銀染色し、目的のスポットを Ettan Spot Picker により切り出した後，MALDI-TOFMS：AXIMA-CFR plus を用いて解析、得られた Peptide mass fingerprint を Mascot データベースにより解析した。Mascot で得られた結果と Swiss-2DPAGE のデーターが一致した、または複数回の MALDI-TOFMS→Mascot 解析により結果が再現された場合に同定したと判定した。さらに臨床データーと比較して、実薬群に特異的に変動し臨床効果と相関する蛋白を同定した。同定した蛋白の RNA を RT-PCR で増幅してベクターに組み込み、蛋白発現系でリコンビナントを作成した。作成したリコンビナント蛋白を IgE、ケモカイン産生、サイトカイン産生においてどのように影響をおよぼすか検討した。

C. 研究結果

オープン試験として舌下免疫療法 1 年目 16 名、2 年目 34 名、3 年目 34 名、4 年目 14 名で治療効果の検討を行った。その結果、舌下免疫療法継続 2 年目において、1 年目に比較し有意に症状と薬物使用の改善を認めた。免疫治療前の花粉症症状を対照とした VAS では、1 年目で 52%、2 年目で 65%、3 年目で 70%、4 年目で 82% の症状軽減となり 3 年目と 4 年目が 1 年に比較して有意差を認めた。これらの結果から、有意差は出ずとも、舌下免疫療法の回数は多い方が良く、舌下免疫療法は最低 2 年間継続する必要性があることが判明した。

スギ花粉飛散ピーク時の薬物スコアが零である割合は、1 年目で 22%、2 年目で 43%、3 年目で 50%、4 年目 60%であり、順次内服が不要となった。総 148 名の治療を行った結果、症状の軽快を認めた患者(VAS で 50%以上の症状軽減)は、70%であった。

平成 20 年度のスギ花粉飛散は、3000 個程度ながら飛散期間が約 2 週間と短かった。週 1 回群 16 名、隔日群 11 名による無作為振り付け 2 群間試験を行ったところ、隔日群では、週 1 回群に比較して症状スコアと薬物スコアの合計で優れていたが、有意差はつかなかった。QOL スコアと visual analog score (VAS)による症状の自己評価ではも、2 群間に有意差は認めなかった。

舌下免疫療法の血清中網羅的蛋白解析では、実薬群特異的に変動する 5 種類の蛋白を同定しえた。そのうち Apolipoprotein A IV (アポ A4) が臨床効果と有意に相関し、症状が改善した者に高い誘導が認められ、改善が乏しかったものでは低値を示していた。リコンビナントアポ A4 は、CryJ1 刺激によるヒト好塩基球のヒスタミン遊離率を抑制した。しかしヒト好酸球の IL-4 産生、ヒト CD4 陽性 T 細胞の IL-5 産生、ヒト好塩基球の IL-13 産生、末梢単核球の IgE 産生には影響を及ぼさなかった。また線維芽細胞の Eotaxin 、GM-CSF 産生にも影響を及ぼさなかった。また二重盲検試験において実薬群では、プラセボ群に対して、in vitro 末梢リンパ球での CryJ1 刺激によるヒスタミン遊離試験においても有意に低値を示した。アポ A4 は、外科にて採取され小腸上皮細胞に認められた。

D. 考察

オープン試験の結果からは、舌下免疫療法を 2 年以上行えば、かなりの症状軽減と内服・点鼻の軽減が得られることが判明した。具体的には 3 年間舌下免疫療法を行えば、約半分の患者が飛散時に内服が不要になり、20 回以上のくしゃみ、20 回以上の擤鼻、口呼吸しかできない重症の鼻閉が、数回（4～6 回）のくしゃみと擤鼻になり、鼻閉は軽度もしくは認めない程度になる。

皮下投与と舌下との二重盲検試験はどこでも行われていないが、実際の臨床所見の改善は、ほぼ同じ程度と考えられる。しかし皮下投与でのアナフュラキシーをはじめとする副反応は舌下ではほとんど認められず、かつ痛みも伴わないことから、この 2 点からも舌下免疫療法の優位性が立証できると考える。

アポ A4 は、HDL や VHDL に結合しそれらの輸送に関連する蛋白である。他のアポ蛋白に比べてほとんど調べられていない。最近、アポ A4 が大腸炎モデルにおいて抗炎症作用を示すことや IL-4 産生や TNF・産生抑制を示すことが報告された。さらに抗酸化作用やデキサメタゾン投与によってもアポ A4 が上昇し、PP・R の活性に関連があることも報告された。またアポ A4 の遺伝子多型解析では、アトピー疾患関連遺伝子多型と有意に相関していることも報告された。酸化コレステロールが T 細胞の活性化に関与するこ

とや、アポ蛋白を介した脂質抗原提示も証明されている。リコンビナントアポA4がCD34陽性細胞からのヒスタミン遊離を抑制したこと、実薬群でヒスタミン遊離率が低下したことは、このアポ A4 が有力なアレルギー性鼻炎の治療薬になりえる可能性が認められた。しかし今後は、遺伝子欠損マウスでのアレルギーマウスモデルなどでの研究を行う必要性があり、現在アレルギーマウスモデル、遺伝子改編マウスでのアポA4の効果とアポA4の標的細胞の同定、その制御機序の解明、効果的な誘導の検討を行っており、臨床使用の実現化に向かっている。

E. 結論

舌下免疫療法は、内服・点鼻・点眼の使用を有意に低下させ、臨床症状を改善する、根治的治療法であるといえる。最低2年の治療が必要であるが、現在の治療状態から計算すると年間6回程度の受診でよく、かつ自宅で行える治療法である。アポA4は、舌下免疫療法で効果を示した症例で上昇していた。リコンビナントアポA4は、CryJ1刺激によるヒト好塩基球のヒスタミン遊離率を抑制し、舌下免疫療法で効果を認めた群での末梢リンパ球でのCryJ1刺激によるヒスタミン遊離試験低値と一致していた。これらのことは、アポA4によるアレルギー治療の可能性を示すとともに舌下免疫療法の治療効果予測の指標としての有用性が示唆される。

F. 健康危険情報

なし

G. 研究発表

1．論文発表

1) Okubo K, Gotoh M, Fujieda S, Okano M, Yoshida H, Morikawa H, Masuyama K, Okamoto Y, Kobayashi M. A randomized double-blind comparative study of sublingual immunotherapy for cedar pollinosis. Allergol Int. 2008 Sep;57(3):265-275.
2) Enomoto H, Hirata K, Otsuka K, Kawai T, Takahashi T, Hirota T, Suzuki Y, Tamari M, Otsuka F, Fujieda S, Arinami T, Noguchi E. Filaggrin null mutations are associated with atopic dermatitis and elevated levels of IgE in the Japanese population: a family and case-control study. J Hum Genet. 2008;53(7):615-621.
3) Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, Fujieda S, Nakamura Y, Yasuda K, Nakanishi K and Tamari M. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. Clin Exp Allergy, 2008;38:1875-1881.
4) 坂下雅文、広田　朝光、藤枝　重治　アレルギー性鼻炎　アレルギー・免疫　15;40-48, 2008
5) 藤枝重治　花粉症の診断　日医雑誌 136(10);1971-1974, 2008.
6) 藤枝重治 職業性鼻炎　耳展　51(6);8-23, 2008
7) 藤枝重治、秋山一男、下条直樹　上気道・下気道のアレルギー　その関連と年齢の影響 呼吸 27(8) 764-775, 2008

2．学会発表

1) Shigeharu Fujieda and Hideyuki Yamamoto. Platelet Derived Endothelial Cell Growth Factor/Thymidine Phosphorylase Enhanced Human IgE Production. AAAAI 2008 annual meeting. 2008.3. Philadelphia
2) 藤枝重治　スギ花粉症における網羅的遺伝子・蛋白解析　第20回日本アレルギー学会 2008.6. 東京
3) 大澤陽子、高橋昇、窪誠太、小嶋章弘、山本英之、山田武千代、藤枝重治 スギ特異的舌下免疫療法の治療効果の検討 第47回日本鼻科学会総会 2008.9. 名古屋
4) 藤枝重治　アレルギー性鼻炎を考える:遺伝子・蛋白解析から治療まで　第58回日本アレルギー秋季学術大会　2008.11.　東京
5) 意元義政、野口恵美子、有波忠雄、坂下雅文、広田朝光、玉利真由美、藤枝重治 フィラグリン機能喪失変異とスギ花粉症の関連解析 第58回日本アレルギー学会秋季大会 2008.11.　東
6) 坂下雅文、広田朝光、大澤陽子、人見裕基、原田通成、玉利真由美、藤枝重治 酸化ストレス関連遺伝子(CYP1A1)の成人スギ花粉症

における影響 第58回日本アレルギー学会秋季学術大会 2008.11. 東京

7) 大澤陽子、高橋昇、窪誠太、小嶋章弘、山本英之、山田武千代、藤枝重治 スギ特異的舌下免疫療法の治療効果 第59回日本アレルギー学会秋季学術大会 2008.11. 東京

8) 大澤陽子、高橋昇、窪誠太、小嶋章弘、山本英之、山田武千代、藤枝重治 スギ特異的舌下免疫療法の治療効果の検討 第311回日本耳鼻咽喉科学会北陸地方部会連合会 2008.12. 金沢

H. 知的財産権の出願・登録状況（予定を含む）

１．特許取得

藤枝重治、高橋　昇、大澤　陽子、窪　誠太、有波 忠雄、野口　恵美子、牧野　友香、内田　和彦、大久保　公裕　アレルギー疾患の治療薬且つ治療効果マーカー（特願2008－053768　平成20年3月4日提出）

２．実用新案登録

なし

３．その他

なし

J Hum Genet (2008) 53:615–621
DOI 10.1007/s10038-008-0293-z

ORIGINAL ARTICLE

Filaggrin null mutations are associated with atopic dermatitis and elevated levels of IgE in the Japanese population: a family and case–control study

Hisako Enomoto · Kenji Hirata · Kenta Otsuka · Toshiharu Kawai · Takenori Takahashi · Tomomitsu Hirota · Yoichi Suzuki · Mayumi Tamari · Fujio Otsuka · Shigeharu Fujieda · Tadao Arinami · Emiko Noguchi

Received: 24 December 2007 / Accepted: 17 March 2008 / Published online: 3 June 2008

Abstract Filaggrin (*FLG*) plays an important role in the barrier function of the skin. Several loss-of-function mutations in the *FLG* gene have been identified in patients with ichthyosis vulgaris, and these null mutations are associated with atopic dermatitis (AD) development. In this study, we examined tag single nucleotide polymorphisms (tSNPs) and null mutations in *FLG* for possible associations with AD and atopic phenotypes in a Japanese population. Transmission disequilibrium test of 105 AD families showed that the null allele of the S2554X variant of *FLG* tended to be overtransmitted to AD-affected offspring; however, the *P* value did not reach statistical significance. In a case–control comparison of 376 AD cases and 923 nonallergic controls, the null allele of S2554X was significantly associated with AD ($P = 0.0012$), and the association was strengthened in subjects with AD alone ($P = 0.000024$). We found that 3321delA and S2554X were also associated with elevated levels of immunoglobulin E (IgE). Combined null mutation carriers were observed more in AD patients and in subjects with high IgE than in control subjects. The combined *P* value for the family and case–control data was significant for the S2554X and combined null mutations. Our data further support the importance of *FLG* in AD development.

Keywords Filaggrin · Atopic dermatitis · Null mutations · Ichthyosis vulgaris · IgE

H. Enomoto · K. Hirata · K. Otsuka · T. Kawai · T. Arinami · E. Noguchi (✉)
Department of Medical Genetics,
Graduate School of Comprehensive Human Sciences,
University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, Japan
e-mail: enoguchi@md.tsukuba.ac.jp

H. Enomoto · T. Takahashi · F. Otsuka
Department of Dermatology,
Graduate School of Comprehensive Human Sciences,
University of Tsukuba, Tsukuba, Ibaraki, Japan

T. Hirota · M. Tamari
Laboratory of Genetics of Allergic Disease,
RIKEN SNP Research Center, Yokohama, Japan

Y. Suzuki
Department of Public Health,
Chiba University Graduate School of Medicine,
Chiba, Japan

S. Fujieda
Departments of Otorhinolaryngology and Immunology,
University of Fukui Faculty of Medical Sciences, Fukui, Japan

Introduction

Atopic dermatitis (AD) is an itchy, chronic, inflammatory skin disease categorized as an atopic disease, along with atopic asthma and rhinitis. The prevalence of AD has been studied in a wide variety of populations, and its reported frequency ranges from 0.73% to 23% (Levy et al. 2003). The 12-month prevalence of symptoms of atopic eczema in Japanese children 6–7 years of age was 16.9%, the second highest after Sweden (Williams et al. 1999). Twin and family studies have indicated that predisposition for AD is highly heritable (Larsen et al. 1986), with a heritability value of 0.72 (Nystad et al. 2005). However, details regarding inheritance of AD remain unclear.

To identify susceptibility genes for AD, genome-wide linkage studies and candidate gene approaches have been used. To date, five genome-wide linkage studies have been performed in Caucasian populations (Lee et al. 2000; Cookson et al. 2001; Bradley et al. 2002; Haagerup et al. 2004) and a Japanese population (Enomoto et al. 2007),

and evidence for linkage to AD was obtained for several chromosomal regions. Several candidate genes, mainly immune-related genes including interleukin (IL)-4 (*IL4*), *IL13*, *IL5*, *IL12B*, and serine protease inhibitor Kazal-type 5 (*SPINK5*) have been examined for possible association with AD (Morar et al. 2006). Recent studies have emphasized the importance of skin-barrier function in AD development. Loss-of-function mutations in the filaggrin gene (*FLG*) were found to be associated with AD in independent populations (Irvine 2007). FLG protein is present in the granular layers of the epidermis, and the keratohyalin granules in the granular layers are predominantly composed of the 400-kDa polyprotein, profilaggrin (Dale et al. 1985; Listwan and Rothnagel 2004; Candi et al. 2005). On the differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into 10–12 essentially identical 37-kDa filaggrin peptides. FLG proteins aggregate the keratin cytoskeleton system to form a dense protein-lipid matrix that is crosslinked by transglutaminases to form the cornified cell envelope (Candi et al. 2005). This structure prevents epidermal water loss and impedes the entry of allergens, toxic chemicals, and infectious organisms. Therefore, *FLG* is a key protein in terminal differentiation of the epidermis and skin-barrier function (Gan et al. 1990).

FLG is located on human chromosome 1q21 (Compton et al. 2002), for which a previous genome-wide linkage study found evidence of linkage with AD (Cookson et al. 2001). The chromosome 1q21 region harbors the epidermal differentiation complex (EDC), which is a dense cluster of genes involved in the terminal differentiation of the epidermis and formation of the stratum corneum, the outermost dead cell compartment of the skin where the main skin barrier function occurs (Mischke et al. 1996). *FLG* is located in the EDC. Recently, we performed a genome-wide linkage analysis of Japanese families with AD and found weak evidence for linkage on 1q24 (Enomoto et al. 2007) near 1q21.

FLG was initially identified as a susceptibility gene for ichthyosis vulgaris (Smith et al. 2006), a disorder of keratinization, and Palmer et al. (2006) reported that two nonsense mutations in *FLG*—R501X and 2282del4—were associated with AD development in a Caucasian population. These mutations showed a combined allele frequency of ~4% in populations of European ancestry, and the variants were greatly overrepresented in the cohort with AD, indicating a highly significant dominant risk of AD (Palmer et al. 2006). *FLG* null alleles X501 and 2282del4 occur at higher frequency in individuals with both asthma and AD than in individuals with asthma alone (Palmer et al. 2006). The R501X and 2282del4 variants were absent in non-European populations, such as those of Asian or African origin.

The associations of *FLG* null alleles with AD development have been replicated in several European populations (Marenholz et al. 2006; Ruether et al. 2006; Sandilands et al. 2006; Weidinger et al. 2006; Barker et al. 2007; Stemmler et al. 2007; Weidinger et al. 2007). *FLG* null alleles were also found to be associated with elevated levels of immunoglobulin E (IgE) (Weidinger et al. 2006, 2007) and allergic sensitization (Weidinger et al. 2006), and Marenholz et al. (Marenholz et al. 2006) reported that those mutations predispose carriers to asthma, allergic rhinitis, and allergic sensitization only in the presence of AD. Other *FLG* null mutations have also been found to be associated with AD in a Caucasian population (Sandilands et al. 2007). In a Japanese population, the 3321delA and S2554X mutations were associated with ichthyosis vulgaris and AD (Nomura et al. 2007). Most of the previous studies related to *FLG* mutations were conducted with European populations, and studies of *FLG* variants other than the null mutations in relation with AD have not been conducted.

In this study, we examined tag single nucleotide polymorphisms (tSNPs) and null mutations in *FLG* for possible associations with AD and atopic phenotypes in a Japanese population.

Materials and methods

Subjects

Probands in the AD families were patients with AD who visited the Dermatology Department of the University Hospital of Tsukuba (Japan) and dermatology departments of several hospitals in Ibaraki, Japan. AD was diagnosed in subjects according to the criteria of Hanifin and Rajka (1980). All patients had pruritus, typical appearance of AD, and a tendency toward chronic or chronically relapsing dermatitis. A full verbal and written explanation of the study was given to all family members interviewed, and 105 families (381 members) gave informed consent and participated in this study. The mean age of the probands and their siblings was 13.3 years (range 0.9–42 years). For a case–control study, 376 independent AD patients (ages 16–64 years, mean 29.7 years) were recruited. Control subjects for the case–control study were 923 healthy adults (ages 19–78 years, mean 46.2 years) with no history of any allergic disease. A full verbal and written explanation of the study was given to patients and all family members interviewed, and all provided informed consent. This study was approved by the Committee of Ethics of the University of Tsukuba. The subjects for the case–control study were classified according to AD alone, elevated total serum IgE level (>1,000 IU/l), and early onset (<2 years of age). Among 376 patients with AD, the number of patients with

AD alone (i.e., AD patients without another atopic disease such as asthma and rhinitis) was 75 (20%). The number with an elevated total serum IgE was 212 (56%), and the number with early onset was 112 (30%).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes or oral brushed cells using standard protocol. R501X and 2282del4 were genotyped by restriction enzyme digestion of polymerase chain reaction (PCR) products amplified from DNAs of 96 unrelated Japanese AD patients. The R501X and 2282del4 variants were PCR amplified with the following primer sequences, 5′-CTGGAGGAAGACA AGGATCG-3′ and 5′-TTGTCTGCTTGCACTTCTGG-3′ for the R501X and 5′-ATCAGGCACTCGTCACACAC-3′ and 5′-AGTGCCTGGAGTTGTCTCGT-3′ for 2282del4. PCR products were digested with *Nla*III for R501X and *Dra*III for 2282del4 at 37°C for 16 h. Digested PCR fragments were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining and ultraviolet transillumination. Expected product sizes for R501 were 213 and 32 bp and for X501 allele were 177, 36, and 32 bp. Expected product sizes for the wild-type allele of 2282del4 were 458 bp, and for the deletion allele were 240 and 214 bp. We genotyped 3321delA with sizing of a fluorescently labeled PCR fragment on an Applied Biosystems 3100 DNA Sequencer (Foster City, CA, USA) as described previously (Nomura et al. 2007). Genotype information for the *FLG* region in Asian populations (Japanese and Chinese) was downloaded from the HapMap database (http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B36/), and tSNPs were selected with Tagger software (de Bakker et al. 2005) implemented in Haploview software (Barrett et al. 2005) with an r^2 threshold of 0.8 and allele frequencies of 0.05. Tag SNPs (rs11582620, rs11586114, rs1933064, rs2065958, rs3814299, rs12730241) were genotyped with TaqMan Assay-on-Demand™ SNP Typing Systems (Applied Biosystems). We genotyped S2554X on a TaqMan Assay-by-Design system for SNP genotyping (Applied Biosystems), with the following primer sequences: forward, 5′-CGGCTCCAGGCACTCA-3′, reverse, 5′-ATCCCCAG TTCCTGCTTGTC-3′ reporter 1 (VIC), 5′-CCCCTCTGA TTGTC-3′ and reporter 2 (FAM), 5′-CCCCTCTCATTG TC-3′. Genotyping accuracy was confirmed based on the direct sequences of samples obtained from carriers and noncarriers of the S2554X null mutation.

Statistical analysis

Transmission disequilibrium test (TDT) and pedigree disequilibrium test (PDT) were performed with the unphased program (http://www.mrc-bsu.cam.ac.uk/personal/frank/software.unphased/). Linkage disequilibrium (LD) between SNPs, as expressed by D', was calculated with Haploview software (Barrett et al. 2005). The significance of differences in the allele frequencies between case and control groups in case–control comparisons was determined by the χ^2 test. To combine family and case–control data, control alleles in AD families were constructed as nontransmitted parental allele and case alleles as transmitted parental alleles as described by Kirov et al. (Kirov et al. 1999).

Results

The X501 and 2282del4 alleles were not identified in 96 independent Japanese patients with AD. The allele frequencies for all SNPs in parents in AD families and in controls did not deviate from Hardy-Weinberg equilibrium predictions ($P > 0.05$). TDT revealed that the minor alleles of rs2065958 and rs12730241 were overtransmitted to AD-affected offspring ($P < 0.05$, Table 1). However, these results were not replicated in the AD case–control study (Table 2). In the AD case–control study, we genotyped two nonsynonymous SNPs, rs2065958 and rs3814299, because rs12730241 is in nearly complete LD with rs2065958 ($r^2 = 0.95$). The null allele of S2554X tended to be overtransmitted to AD-affected offspring, though the P value

Table 1 Transmission disequilibrium test (TDT) and pedigree disequilibrium test (PDT) analysis of the *FLG* polymorphisms in Japanese atopic dermatitis (AD) families

SNP single nucleotide polymorphism, *T* number of alleles transmitted to affected children, *NT* number of alleles not transmitted to affected children

Polymorphism	SNP	Allele	Allele frequency	T	NT	TDT *P* value	PDT *P* value
rs11582620	A/G	A	0.89	41	26	0.066	0.059
rs11586114	A/G	G	0.55	90	78	0.35	0.11
rs1933064	A/G	A	0.85	44	35	0.31	0.24
rs2065958(D3105Y)	A/C	C	0.36	69	92	0.069	0.038
rs3814299(L3970S)	A/G	A	0.63	95	70	0.24	0.38
rs12730241	A/G	G	0.057	21	14	0.051	0.021
3321delA	A/–	del	0.014	4	4	1	1
S2554X	C/G	G	0.021	10	4	0.11	0.16

Table 2 Case–control study for atopic dermatitis (AD) in *FLG* polymorphisms

Polymorphism	Population[a]	Genotype count (frequency%)			Genotypic P^b	Odds ratio (95% CI)[c]	Allelic P^b
		C/C	C/A	A/A			
rs2065958	AD	58 (15.6)	156 (41.9)	158 (42.5)	0.94	1.0 (0.8–1.2)	0.84
	Only AD	9 (12.3)	25 (34.3)	39 (53.4)	0.14	0.6 (0.4–1.0)	0.068
	IgE > 1,000 IU/l	30 (14.2)	90 (42.9)	90 (42.9)	0.89	1.0 (0.7–1.3)	0.62
	Early onset	12 (10.7)	57 (50.9)	43 (38.4)	0.20	1.1 (0.8–1.7)	0.81
	Controls	142 (15.5)	393 (43.0)	380 (41.5)			
		A/A	A/G	G/G			
rs3814299	AD	2 (0.5)	52 (13.8)	322 (85.7)	0.64	1.1 (0.8–1.6)	0.54
	AD alone	0 (0)	12 (16.0)	63 (84.0)	0.46	1.3 (0.7–2.5)	0.57
	IgE > 1,000 IU/l	1 (0.5)	34 (16.1)	176 (83.4)	0.26	1.4 (0.9–2.0)	0.21
	Early onset	1 (0.9)	14 (12.5)	97 (86.6)	0.98	1.1 (0.6–1.9)	0.84
	Controls	7 (0.8)	111 (12.0)	804 (87.2)			
		A/A	A/–	–/–			
3321delA	AD	356 (97.3)	10 (2.7)	0 (0)	0.077	2.1 (0.9–4.9)	0.077
	AD alone	71 (95.9)	3 (4.1)	0 (0)	0.064	3.2 (0.9–11.5)	0.064
	IgE > 1,000 IU/l	198 (95.6)	9 (4.4)	0 (0)	0.0038	3.4 (1.4–8.2)	0.0036
	Early onset	103 (97.2)	3 (2.8)	0 (0)	0.22	2.2 (0.6–7.9)	0.22
	Controls	902 (98.7)	12 (1.3)	0 (0)			
		C/C	C/G	G/G			
S2554X	AD	365 (97.3)	10 (2.7)	0 (0)	0.0012	5.0 (1.7–14.8)	0.0012
	AD alone	71 (94.7)	4 (5.3)	0 (0)	0.000024	10.3 (2.7–39.4)	0.000024
	IgE > 1,000 IU/l	207 (97.6)	5 (2.3)	0 (0)	0.011	4.4 (1.3–15.5)	0.011
	Early onset	110 (98.2)	2 (1.8)	0 (0)	0.13	3.3 (0.6–17.4)	0.13
	Controls	918 (99.5)	5 (0.5)	0 (0)			
Combined		Wild allele/wild allele	Wild/at least one null allele	Null/null			
(3321delA and S2554X)	AD	355 (64.7)	20 (5.3)	0 (0)	0.00073	3.0 (1.5–5.8)	0.00067
	AD alone	67 (90.5)	7 (9.5)	0 (0)	0.000047	5.5 (2.2–13.8)	0.000042
	IgE > 1,000 IU/l	198 (93.4)	14 (6.6)	0 (0)	0.00015	3.7 (1.8–7.7)	0.00014
	Early onset	101 (95.3)	5 (4.7)	0 (0)	0.054	2.6 (1.0–7.3)	0.056
	Controls	900 (98.1)	17 (1.9)	0 (0)			

CI confidence interval, *IgE* immunoglobulin E

[a] AD alone; AD patients without other atopic disease. Early onset; patients whose age at disease onset was younger than 2 years

[b] Genotypic *P* and allelic *P* values were calculated with χ^2 test in comparison with genotype and allele counts in controls, respectively

[c] Odds ratio for the wild type homozygote versus minor allele heterozygote and minor allele homozygote

did not reach statistical significance. In the case–control comparison, the null allele of S2554X was associated statistically significantly with AD (Table 2). S2554X was also associated with high IgE levels and the phenotype of patients with AD alone. Five percent of patients with the phenotype with AD alone carried the S2554X null mutation, whereas only 1% of healthy control subjects had the null mutation ($P = 2.4 \times 10^{-5}$). Three percent of AD patients and 4% of those with the phenotype of patients with AD alone carried the 3321delA allele, whereas 1% of healthy control subjects had the null mutation. However, this difference was not statistically significant ($P > 0.05$). Association was observed between 3321delA and the high-IgE phenotype ($P = 0.0036$). Combined null mutation carriers (subjects carrying either X2554 or 3321delA alleles) were observed more in patients with the AD and high-IgE phenotypes than in control subjects. The most significant association was observed for the phenotype of patients with AD alone (seven of 67 patients, carrier frequency 9.5%, $P = 4.2 \times 10^{-5}$). Subjects with compound heterozygous null mutations were not observed in our family or case–control samples.

To combine the TDT and case–control data, the proband of each family was selected, and an artificially constructed

Table 3 Combined *P* values of *FLG* polymorphisms in families and case–control study

Polymorphism	Genotype count (frequency)				Genotypic P^a	Odds ratio (95% CI)[b]	Allelic P^a
		C/C	C/A	A/A			
rs2065958	AD	65 (13.8)	202 (43.0)	203 (43.2)	0.54	0.92 (0.7–1.1)	0.28
	Controls	161 (15.9)	437 (43.0)	417 (41.1)			
		A/A	A/G	G/G			
rs12730241	AD	4 (0.8)	60 (12.6)	412 (86.6)	0.91	1.1 (0.8–1.5)	0.69
	Controls	7 (0.7)	124 (12.1)	892 (87.2)			
		A/A	A/–	–/–			
3323delA	AD	454 (97.4)	12 (2.6)	0 (0)	0.14	1.8 (0.8–3.8)	0.14
	Controls	999 (98.5)	15 (1.5)	0 (0)			
		C/C	C/G	G/G			
S2554X	AD	459 (97.6)	16 (3.4)	0 (0)	0.000091	5.0 (2.1–12.3)	0.0001
	Controls	1010 (99.3)	7 (0.7)	0 (0)			
Combined		Wild/wild	Wild/null	Null/null			
(3321delA and S2554X)	AD	438 (94.0)	28 (6.0)	0 (0)	0.00015	2.9 (1.6–5.1)	0.00017
	Controls	992 (97.8)	22 (2.2)	0 (0)			

CI confidence interval

[a] Genotypic *P* and allelic *P* values were calculated with χ^2 test in comparison with genotype and allele counts in controls, respectively

[b] Odds ratio for the wild type homozygote versus minor allele heterozygote and minor allele homozygote

case population consisting of parental alleles transmitted to the affected child and a control population of nontransmitted alleles in the AD family trios were determined (Kirov et al. 1999). These "cases" and "controls" in the family trios were combined with the genotype data (Table 3). The combined *P* value was significant for the S2554X polymorphism and null mutations of *FLG* ($P = 0.0001$), whereas rs2065958 and rs12730241 were not associated with AD development.

Discussion

In this study, we found that the null allele of S2554X was associated with AD development, confirming previous studies showing that *FLG* null mutations are associated with AD (Nomura et al. 2007). Our study found 1% of healthy subjects without any allergic diseases carried *FLG* null mutations, whereas a pervious study found no control subjects carried the null mutations (Nomura et al. 2007). Allele frequency of *FLG* null mutations in AD patients were similar to those reported previously (Nomura et al. 2007). Null alleles of R501X and 2282del4 were not detected in 96 Japanese AD subjects.

FLG is thought to be one of the most important factors in skin-barrier function. In children, dry skin is often the earliest sign of AD. Impairment of epidermal-barrier function is a clinical hallmark of AD. Microarray analysis revealed decreased expression of *FLG* messenger ribonucleic acid (mRNA) in active atopic skin (Sugiura et al. 2005). These findings suggest that dysfunction of *FLG* is an important factor in AD development. In our study, the most significant effect of *FLG* null mutations was observed in the phenotype of patients with AD alone. AD patients often suffer from other atopic diseases, such as asthma and allergic rhinitis, and patients with multiple atopic diseases exhibit increased levels of IgE against allergens. AD patients suffering from other atopic diseases are more likely to exhibit allergic skin inflammation, which leads to AD development. In contrast, because FLG plays an important role in skin-barrier function, the skin may be fragile in carriers of the *FLG* null allele, regardless of the atopic status of these individuals. This may result in the development of AD. Therefore, subjects with the phenotype of AD alone are more likely to carry the *FLG* null allele than those with the phenotype of AD along with other atopic diseases.

The study by Palmer et al. (2006) was the first to show that *FLG* null mutations are associated with AD in Caucasian populations. A number of studies have been conducted to replicate the original findings, and some have confirmed and others refuted the association of *FLG* with AD (Marenholz et al. 2006; Ruether et al. 2006; Weidinger et al. 2006, 2007; Barker et al. 2007; Morar et al. 2007; Stemmler et al. 2007). To examine the association of common *FLG* variants with AD development, we performed tSNP analysis of Japanese AD families and case–control subjects. Two SNPs, including one nonsynonymous mutation, were associated with AD by PDT analysis, but this finding was not confirmed in case–control subjects. The statistical

power of the case–control study for these SNPs was more than 80% at the alpha level of 0.05 if the relative risk for AD in those persons carrying a putative risk allele was 1.5 compared with that in persons without the allele. Therefore, our number of case–control samples was sufficient to detect alleles confirming moderate risk but may not have been sufficient to detect alleles with weak risk.

The results of our family-based association study of S2554X did not reach statistical significance. However, the null allele of S2554X tended to be overtransmitted to affected offspring in our Japanese AD families. In the case–control comparison, X2554 was significantly associated with AD development, and the combined *P* value for the family and case–control data was significant. Because of the low allele frequencies of the null alleles in *FLG*, failure to find an association in the family samples was due to low statistical power. The other null allele, 3321delA, was not associated with AD. The allele frequency of 3321delA was very low: 2.7% in AD patients and 1.3% in control. Statistical power in the pedigree samples was <0.1 at the alpha level of 0.05 if the relative risk for AD in those persons carrying a putative risk allele was 2.0 compared with that in persons without the allele. In the case–control study, 567 cases would be required to achieve statistical power of 0.8 at the alpha level of 0.05 if the relative risk for AD in those persons carrying a putative risk allele was 2.0 compared with that in persons without the allele. Therefore, our sample size was not enough to assess the genotypic relative risk <2. However, combined analysis of the *FLG* 3321delA and S2554X null mutations showed significant association with AD. R501X and 2282del4 were the first null mutations reported to be associated with AD in a European population (Irvine 2007), and a subsequent study identified three additional null alleles of *FLG* (R2447X, S3247X, 3702delG) associated with development of AD (Sandilands et al. 2007). These three null mutations were not found in an Asian population (Sandilands et al. 2007), whereas the3321delA and S2554X null alleles were not found in a European population (Sandilands et al. 2007). Our tSNP analysis included common missense mutations (D3105Y and L3970S), but the results were not consistent across family and case–control data. *FLG* null mutations were also associated with high IgE levels. Allergens can penetrate through the skin, leading to allergic sensitization in susceptible individuals. Skin-barrier dysfunction may accelerate allergen penetration, and therefore, loss of *FLG* function can contribute to allergic sensitization and the high-IgE phenotype.

In conclusion, *FLG* null alleles, not common variants, are associated with AD development and high IgE levels in Japanese, confirming the importance of null mutations in *FLG* for disease onset and allergic sensitization in AD patients.

Acknowledgments We are grateful to Dr. Takako Takase (Takase Dermatological Clinic, Tsukuba, Japan) and Dr. Taro Mochizuki (Oho Dermatological Clinic, Tsukuba-City, Japan), Dr. Yoshihiro Nanno (Taga General Hospital, Japan), Dr. Kazuhito Hayakawa, Dr. Tetsuo Shiohara (Kyorin University School of Medicine, Japan) who provided samples and clinical data. This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Health and Welfare, Japan (H17-Genome-001, EN and TA).

References

Barker JN, Palmer CN, Zhao Y, Liao H, Hull PR, Lee SP, Allen MH, Meggitt SJ, Reynolds NJ, Trembath RC, McLean WH (2007) Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol 127:564–567

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Bradley M, Soderhall C, Luthman H, Wahlgren CF, Kockum I, Nordenskjold M (2002) Susceptibility loci for atopic dermatitis on chromosomes 3, 13, 15, 17 and 18 in a Swedish population. Hum Mol Genet 11:1539–1548

Candi E, Schmidt R, Melino G (2005) The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 6:328–340

Compton JG, DiGiovanna JJ, Johnston KA, Fleckman P, Bale SJ (2002) Mapping of the associated phenotype of an absent granular layer in ichthyosis vulgaris to the epidermal differentiation complex on chromosome 1. Exp Dermatol 11:518–526

Cookson WO, Ubhi B, Lawrence R, Abecasis GR, Walley AJ, Cox HE, Coleman R, Leaves NI, Trembath RC, Moffatt MF, Harper JI (2001) Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. Nat Genet 27:372–373

Dale BA, Resing KA, Lonsdale-Eccles JD (1985) Filaggrin: a keratin filament associated protein. Ann N Y Acad Sci 455:330–342

de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D (2005) Efficiency and power in genetic association studies. Nat Genet 37:1217–1223

Enomoto H, Noguchi E, Iijima S, Takahashi T, Hayakawa K, Ito M, Kano T, Aoki T, Suzuki Y, Koga M, Tamari M, Shiohara T, Otsuka F, Arinami T (2007) Single nucleotide polymorphism-based genome-wide linkage analysis in Japanese atopic dermatitis families. BMC Dermatol 7:5

Gan SQ, McBride OW, Idler WW, Markova N, Steinert PM (1990) Organization, structure, and polymorphisms of the human profilaggrin gene. Biochemistry 29:9432–9440

Haagerup A, Bjerke T, Schiotz PO, Dahl R, Binderup HG, Tan Q, Kruse TA (2004) Atopic dermatitis—a total genome-scan for susceptibility genes. Acta Derm Venereol 84:346–352

Hanifin J, Rajka G (1980) Diagnostic feature of atopic dermatitis. Acta Derm Venereol (Suppl) 92:44–47

Irvine AD (2007) Fleshing out filaggrin phenotypes. J Invest Dermatol 127:504–507

Kirov G, Jones I, McCandless F, Craddock N, Owen MJ (1999) Family-based association studies of bipolar disorder with candidate genes involved in dopamine neurotransmission: DBH, DAT1, COMT, DRD2, DRD3 and DRD5. Mol Psychiatry 4:558–565

Larsen FS, Holm NV, Henningsen K (1986) Atopic dermatitis. A genetic-epidemiologic study in a population-based twin sample. J Am Acad Dermatol 15:487–494

Lee YA, Wahn U, Kehrt R, Tarani L, Businco L, Gustafsson D, Andersson F, Oranje AP, Wolkertstorfer A, v Berg A, Hoffmann

U, Kuster W, Wienker T, Ruschendorf F, Reis A (2000) A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. Nat Genet 26:470–473

Levy RM, Gelfand JM, Yan AC (2003) The epidemiology of atopic dermatitis. Clin Dermatol 21:109–115

Listwan P, Rothnagel JA (2004) Keratin bundling proteins. Methods Cell Biol 78:817–827

Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, Gruber C, Lau S, Worm M, Keil T, Kurek M, Zaluga E, Wahn U, Lee YA (2006) Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 118:866–871

Mischke D, Korge BP, Marenholz I, Volz A, Ziegler A (1996) Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ("epidermal differentiation complex") on human chromosome 1q21. J Invest Dermatol 106:989–992

Morar N, Cookson WO, Harper JI, Moffatt MF (2007) Filaggrin mutations in children with severe atopic dermatitis. J Invest Dermatol 127:1667–1672

Morar N, Willis-Owen SA, Moffatt MF, Cookson WO (2006) The genetics of atopic dermatitis. J Allergy Clin Immunol 118:24–34 (quiz 35–26)

Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K, Ota M, Sugiura H, Yamamoto K, Sato H, Palmer CN, Smith FJ, McLean WH, Shimizu H (2007) Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Allergy Clin Immunol 119:434–440

Nystad W, Roysamb E, Magnus P, Tambs K, Harris JR (2005) A comparison of genetic and environmental variance structures for asthma, hay fever and eczema with symptoms of the same diseases: a study of Norwegian twins. Int J Epidemiol 34:1302–1309

Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, Goudie DR, Sandilands A, Campbell LE, Smith FJ, O'Regan GM, Watson RM, Cecil JE, Bale SJ, Compton JG, DiGiovanna JJ, Fleckman P, Lewis-Jones S, Arseculeratne G, Sergeant A, Munro CS, El Houate B, McElreavey K, Halkjaer LB, Bisgaard H, Mukhopadhyay S, McLean WH (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 38:441–446

Ruether A, Stoll M, Schwarz T, Schreiber S, Folster-Holst R (2006) Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. Br J Dermatol 155:1093–1094

Sandilands A, O'Regan GM, Liao H, Zhao Y, Terron-Kwiatkowski A, Watson RM, Cassidy AJ, Goudie DR, Smith FJ, McLean WH, Irvine AD (2006) Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. J Invest Dermatol 126:1770–1775

Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, Carrick T, Evans AT, Liao H, Zhao Y, Campbell LE, Schmuth M, Gruber R, Janecke AR, Elias PM, van Steensel MA, Nagtzaam I, van Geel M, Steijlen PM, Munro CS, Bradley DG, Palmer CN, Smith FJ, McLean WH, Irvine AD (2007) Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet 39:650–654

Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, Liao H, Evans AT, Goudie DR, Lewis-Jones S, Arseculeratne G, Munro CS, Sergeant A, O'Regan G, Bale SJ, Compton JG, DiGiovanna JJ, Presland RB, Fleckman P, McLean WH (2006) Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 38:337–342

Stemmler S, Parwez Q, Petrasch-Parwez E, Epplen JT, Hoffjan S (2007) Two common loss-of-function mutations within the filaggrin gene predispose for early onset of atopic dermatitis. J Invest Dermatol 127:722–724

Sugiura H, Ebise H, Tazawa T, Tanaka K, Sugiura Y, Uehara M, Kikuchi K, Kimura T (2005) Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. Br J Dermatol 152:146–149

Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, Klopp N, Wagenpfeil S, Zhao Y, Liao H, Lee SP, Palmer CN, Jenneck C, Maintz L, Hagemann T, Behrendt H, Ring J, Nothen MM, McLean WH, Novak N (2006) Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 118:214–219

Weidinger S, Rodriguez E, Stahl C, Wagenpfeil S, Klopp N, Illig T, Novak N (2007) Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J Invest Dermatol 127:724–726

Williams H, Robertson C, Stewart A, Ait-Khaled N, Anabwani G, Anderson R, Asher I, Beasley R, Bjorksten B, Burr M, Clayton T, Crane J, Ellwood P, Keil U, Lai C, Mallol J, Martinez F, Mitchell E, Montefort S, Pearce N, Shah J, Sibbald B, Strachan D, von Mutius E, Weiland SK (1999) Worldwide variations in the prevalence of symptoms of atopic eczema in the international study of asthma and allergies in childhood. J Allergy Clin Immunol 103:125–138

doi: 10.1111/j.1365-2222.2008.03114.x

Clinical and Experimental Allergy, 38, 1875–1881

ORIGINAL PAPER Asthma and Rhinitis

Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis

M. Sakashita*†, T. Yoshimoto‡, T. Hirota*, M. Harada*, K. Okubo§, Y. Osawa†, S. Fujieda†, Y. Nakamura¶, K. Yasuda‡, K. Nakanishi‡ and M. Tamari*

**Laboratory for Genetics of Allergic Diseases, SNP Research Center, RIKEN, Kanagawa, Japan, †Department of Otorhinolaryngology, Fukui Medical University, Matsuoka, Fukui, Japan, ‡Department of Immunology and Medical Zoology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan, §Department of Otolaryngology, Nippon Medical School, Tokyo, Japan and ¶Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan*

Clinical and Experimental Allergy

Correspondence:
Mayumi Tamari, Laboratory for Genetics of Allergic Diseases, SNP Research Center, RIKEN, Kanagawa 230-0045, Japan.
E-mail: tamari@src.riken.jp

Cite this as: M. Sakashita, T. Yoshimoto, T. Hirota, M. Harada, K. Okubo, Y. Osawa, S. Fujieda, Y. Nakamura, K. Yasuda, K. Nakanishi and M. Tamari, *Clinical and Experimental Allergy*, 2008 (38) 1875–1881.

Summary

Background IL-33, an IL-1-like cytokine, is a ligand for IL1RL1, which is an important effector molecule of type 2 T helper responses. Although IL-33/IL1RL1 interaction has been suggested to be important in induction of allergic airway inflammation, serum levels of IL-33 and the genetic influences of the polymorphisms of *IL-33* in human allergic diseases are unclear.

Objective The aim of this study was to examine whether the serum IL-33 level and polymorphisms in *IL-33* are associated with Japanese cedar (JC) pollinosis, the most common form of allergic rhinitis, and a major public health problem, in Japan.

Methods We performed linkage disequilibrium (LD) mapping of the gene using the HapMap database, and two selected tag single nucleotide polymorphisms were genotyped. We conducted an association study of IL-33 (JC pollinosis, $n = 170$; normal controls, $n = 100$) and measured the IL-33 levels in sera of the 270 subjects by ELISA.

Results Serum levels of IL-33 were significantly higher in patients with JC pollinosis ($P = 0.0018$) than in controls. In genetic association analysis, we found a positive association between the polymorphism and JC pollinosis ($P = 0.048$).

Conclusion Our results support a role for IL-33 in the pathogenesis of JC pollinosis.

Keywords association, IL-33, JC pollinosis, polymorphism, serum level
Submitted 28 August 2007; revised 3 February 2008, 31 July 2008; accepted 3 September 2008

Introduction

Allergic diseases are increasing world-wide, and Japanese cedar (JC) pollinosis, which is a disease of allergic rhinitis and allergic conjunctivitis caused by exposure to JC pollen, is one of the most common allergic diseases in Japan [1]. The increase of JC pollinosis in Japan has become a social problem, with a prevalence of > 16% [1]. Allergic rhinitis is the result of an inflammatory reaction triggered by type 2 T helper (Th2) cell-mediated immune responses against allergens [2]. Th2 cytokines induce B cells to produce high amounts of IgG4 and IgE in humans, and promote the growth and differentiation of mast cells and eosinophils [3, 4]. IL1RL1 belongs to the IL-1 receptor family and functions as an important effector molecule of Th2 responses [5–8]. IL-33, an IL-1-like cytokine, has been identified as a ligand for IL1RL1, and can be detected in epithelial cells from the bronchi and small airways, which indicates a possible role in the regulation of mucosal function [9]. It activates NF-κB and mitogen-activated protein kinases, and drives production of Th2-associated cytokines from *in vitro* polarized Th2 cells via IL1RL1 [9]. *In vivo* analysis has demonstrated that IL-33 strongly induces gene expression of Th2-associated cytokines such as IL-4, IL-5 and IL-13, and IL-33-treated mice have significantly higher serum levels of IgE. In addition, IL-33 induces pathological changes in mucosal organs such as the lung, resulting in hypertrophied epithelial lining of the airways with large amount of mucus,

and those changes are primarily restricted to the bronchi and larger bronchioles [9].

Although very little work has been done with IL-33, intensive studies of IL-33 receptor IL1RL1 have shown its regulatory functions in the development and effector phases of Th2 responses [10]. The *IL1RL1* gene encodes a soluble-secreted protein, IL1RL1, and a transmembrane protein, ST2L [11]. In murine models of allergic airway inflammation, increases in endogenous IL1RL1 protein after allergen exposure modulate Th2-mediated airway inflammation [12], and blockade of the binding of the ligand for ST2L using a recombinant IgG fusion protein inhibits allergic inflammation [6, 7]. Other studies have reported that ST2L is a reliable selective marker of both murine and human Th2 lymphocytes in allergic airway inflammation [7, 13]. Moreover, a study has revealed that soluble IL1RL1 acts as a negative regulator of Th2 cytokine production via IL-33 signalling in allergic airway inflammation. In asthmatic patients, serum levels of soluble IL1RL1 are markedly elevated during acute attacks and the magnitude of the elevation correlates with the reduction of pulmonary functions and increased levels of serum IL-5 [14]. These findings imply that IL-33 is a good candidate for involvement in JC pollinosis, an allergen-induced upper airway inflammation.

A large number of association studies using polymorphic markers have been performed to discover genetic components in the pathogenesis of allergic diseases [15–17]. Recently, we have reported that functional single nucleotide polymorphisms (SNPs) in the IL1RL1 distal promoter region are associated with atopic dermatitis. The genetic variants regulate IL1RL1 expression, and immunohistochemical staining of a skin biopsy specimen from an atopic dermatitis patient showed IL1RL1 staining in keratinocytes as well as in cells infiltrating the dermal layer [18]. However, there have been no genetic association studies with IL-33.

In this study, to test whether genetic variations of IL-33 contribute to susceptibility to JC pollinosis, we first selected a genetic polymorphism of IL-33 using HapMap linkage disequilibrium (LD) data and conducted association studies. In addition, we examined the associations between serum IL-33 levels and JC pollinosis and serum total IgE levels.

Methods

Study subjects

All subjects were recruited from residents of Eiheiji-cho, in Fukui prefecture, in the central area of Japan between May and June 2006. Because these participants were workers of the Fukui University hospital and students of nursing and medical colleges in Fukui, the number of females was higher than that of males. Specific IgE to seven aeroallergens, *Cryptomeria japonica*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Candida albicans*, *Aspergillus fumigatus*, *Dactylis glomerata* and *Ambrosia*, were measured with a Pharmacia CAP System (Pharmacia CAP, Uppsala, Sweden) (Table 1). Positive sensitization refers to an allergen-specific serum IgE level >0.7 (CAP RAST score of 2). Diagnosis of JC pollinosis was confirmed by symptoms of allergic rhinoconjunctivitis during the JC pollinosis season and positive serum-specific IgE towards JC pollinosis. A total of 170 patients with JC pollinosis were recruited (Table 1). One hundred healthy subjects who had never had symptoms of allergic rhinitis and showed no sensitization to any of the seven aeroallergens were recruited as controls (Table 1). We recruited 29 subjects with infectious rhinitis who were diagnosed by otolaryngologists and showed no sensitization to any of the seven aeroallergens. All individuals were unrelated Japanese and gave written informed consent to participate in the study according to the rules of the process committees at the School of Medicine, University of Fukui, the Nippon Medical School and The Institute of Physical and Chemical Research.

Selection of polymorphisms for genotyping

Genomic DNA was prepared from peripheral blood samples, using standard protocols. There were 22 SNPs in the *IL-33* gene with a minor allele frequency (MAF) of $>10\%$ in the HapMap Japanese data set (URL: http://www.hapmap.org/index.html.en) (Table 2). Pairwise LD was calculated as r^2 by using the Haploview 3.2 program (http://www.broad.mit.edu/mpg/haploview/). Genotyping of SNPs was performed by the TaqMan™ allele-specific amplification (TaqMan-ASA) method (Applied Biosystems, Foster City, CA, USA). rs1929992 was genotyped by Custom TaqMan® SNP Genotyping Assay Service with primers 5′-GGAAAAAAACACATTTTCCCCCAA-3′ and 5′-AAACCATCTTAACTACTACTTAAAATGTATAAAGTGT TAGAATTAT-3′. The probes used were VIC-TCATGGT CAAAATATTGAAAT and FAM-ATGGTCAAAATGTTGAA AT. rs10975519 was genotyped by TaqMan(R) Pre-Designed SNP Genotyping Assays, C___2762153_10.

Reagents for human interleukin-33

Recombinant human IL-33 (rhIL-33) and a rabbit-neutralizing anti-hIL-33 IgG antibody were made by Hokudo Co., Ltd. (Sapporo, Japan). Briefly, rhIL-33 (mature form) was amplified from human lung cDNA (BioChain Institute, Hayward, CA, USA) as a template, and subcloned into pET28a vector (Novagen, Madison, WI, USA). BL21 (DE3) RIL was transformed and the expressed recombinant protein was purified with Ni-NTA resin. Endotoxin was removed by filtration through Zetapor (Cuno, Meriden, CT, USA). For establishment of a polyclonal antibody to hIL-33, rabbits

were immunized with rhIL-33 (200 μg/body) with CFA, and boosted with rhIL-33 (200 μg/body) with IFA three times every 2 weeks. Seven weeks later, serum was collected and the antibody was purified using a Protein-A sepharose column. This IgG antibody (R2) was further purified with an rhIL-33 sepharose column and was biotinylated with NHS-biotin (Sigma, St Louis, MO, USA) in our laboratory. This purified anti-hIL-33 antibody could completely neutralize 50 ng/mL of IL-33 at the concentration of 10 μg/mL *in vitro*.

Table 1. Characteristics of the patients with Japanese cedar (JC) pollinosis and controls

Characteristics	Case	Control
Age (year, median with range)	30 (20–49)	32.5 (20–49)
Total subjects and sex (% male subjects)	170 (14)	100 (9.0)
Serum total IgE (IU/mL, mean±SEM)	280.2±879.2	42.9±51.5
Atopic sensitization (RAST) (number (%))		
Japanese cedar pollen positive	170 (100)	0 (0)
Dermatophagoides pteronyssinus positive	80 (47.1)	0 (0)
Dermatophagoides farinae positive	78 (45.9)	0 (0)
Candida albicans positive	10 (5.9)	0 (0)
Aspergillus positive	3 (1.8)	0 (0)
Dactylis glomerata positive	61 (35.9)	0 (0)
Ambrosia positive	23 (13.5)	0 (0)

Enzyme-linked immunosorbent assay of serum levels of interleulun-33

To elucidate the biological roles of the *IL-33* gene, we constructed an ELISA system to quantify human IL-33 protein in sera of subjects with JC pollinosis and controls. A 96-well plate (Costar, Cambridge, MA, USA) was coated with the anti-hIL-33 IgG antibody (R2) and blocked with StartingBlock™ blocking buffer (PIERCE, Rockford, IL, USA). Human IL-33 was detected with the biotinylated-anti-IL-33 antibody and streptavidin-HRP. The ELISA system was specific for hIL-33 and did not cross-react with other cytokines tested, which included IL-1β, IL-2, IL-4, IL-12, IL-18, TNF-α, IFN-γ and GM-CSF. Serum samples were collected, and then they were stored at −80 °C until measurement. IL-33 was assayed by ELISA with reference standard curves using known amounts of hIL-33. The lower limit of ELISA sensitivity for serum IL-33 was 30 pg/mL. A value of 0 was assigned to results that were below the assay's lower limit of detection for non-parametric statistical calculations in Fig. 2.

Statistical analysis

We calculated allele frequencies and tested agreement with Hardy–Weinberg equilibrium using a χ^2 goodness-of-fit

Table 2. Locations and allele frequencies of polymorphisms in *IL-33* based on the HapMap JPT data set

SNP*	Location	Amino acid	MAF (%)‡	NCBI§
−5345 G/A	5′-Flanking region		0.477	rs928414
−5194 T/G	5′-Flanking region		0.477	rs4237164
−4432 G/A	5′-Flanking region		0.477	rs10975509
−1611 C/T	5′-Flanking region		0.466	rs7025417
−1037 T/C	Intron 1		0.467	rs10975511
1256 C/T	Intron 1		0.455	rs4742170
2241 C/G	Intron 1		0.455	rs7019575
4450 G/A	Intron 1		0.455	rs10975514
5999 G/A	Intron 1		0.443	rs10975516
9318 C/A	Intron 2		0.443	rs1317230
9813 G/T	Intron 3		0.455	rs1330383
9894 T/C†	Intron 3		0.455	rs1929992
11607 T/C	Intron 4		0.432	rs1113573
11877 C/T†	Exon 5	Tyr163Tyr	0.433	rs10975519
12016 G/C	Intron 5		0.422	rs10975520
12514 T/C	Intron 5		0.427	rs7044343
13206 A/G	Intron 6		0.487	rs7871381
13316 C/A	Intron 6		0.371	rs1412421
13625 G/A	Intron 6		0.422	rs7047921
14187 G/T	Intron 6		0.420	rs1332290
14598 G/A	Exon 7	3′-UTR	0.409	rs1048274
23562 G/C	3′-Flanking region		0.455	rs10815397

*Numbering according to the genomic sequence of IL-33 (AL353741.16) and position 1 is the A of the initiation codon. Major allele/minor allele.
†SNPs were genotyped in this study.
‡Minor allele frequencies
§NCBI, number from the dbSNP of NCBI (http://www.ncbi.nlm.nih.gov/SNP/).
SNP, single nucleotide polymorphisms; MAF, minor allele frequency.

