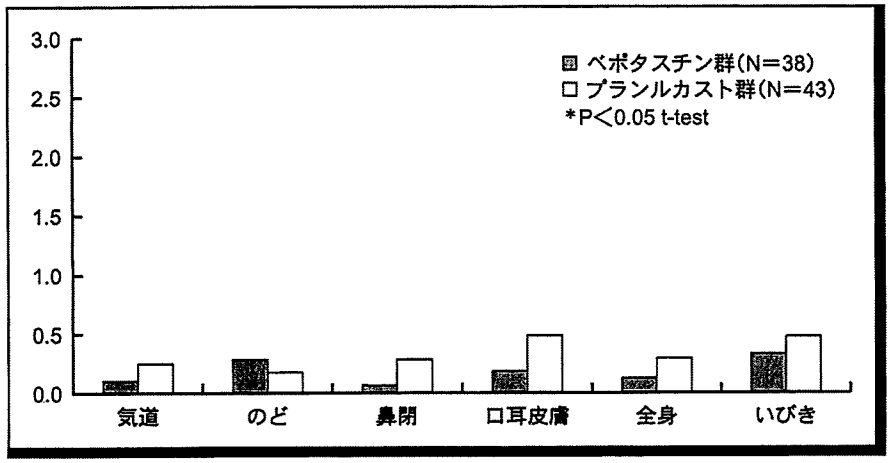

図5　JRQLQ (No.2) スギ花粉飛散初期のサブスケール
両群でサブスケールに有意な差を認めなかった.

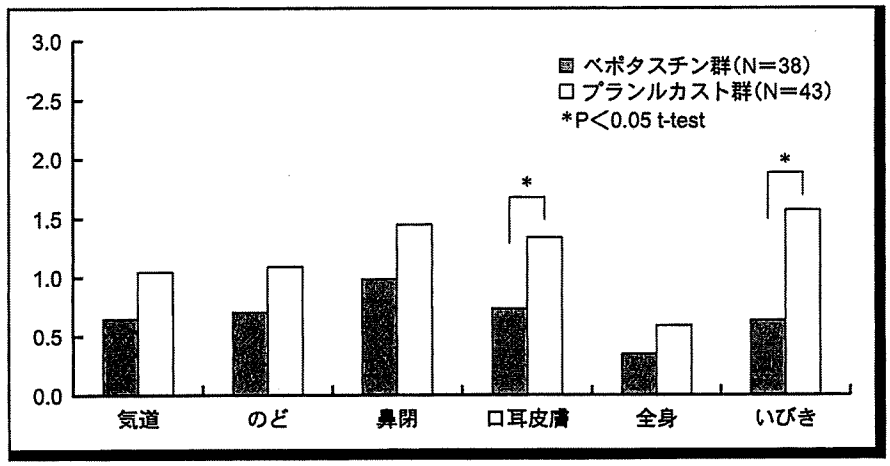

図6　JRQLQ (No.2) スギ花粉飛散ピーク時期のサブスケール
ベポタスチン群ではプランルカスト群と比較して，口耳皮膚およびいびきにおいて，有意なスコアの低下を認めた.

皮膚およびいびきのサブスケールにおいてベポタスチン群で有意なスコアの抑制が認められた（図6）。スギ花粉飛散ピーク時のJRQLQ (No.2) の全項目について，両群のスコアを比較検討すると，鼻づまりと目のかゆみの眼鼻の症状がベポタスチン群で有意に抑制されただけでなく，皮膚のかゆみやいびきなども有意に抑制されていることが確認された（図7）。

3．睡眠障害

スギ花粉症患者の睡眠障害に対するプランルカストとベポタスチンの効果についてPSQIを用いて比較検討した。花粉飛散初期には，両群で明らかな差は認められなかった。しかし，花粉飛散の最初のピーク時期では，ベポタスチン群では睡眠スコアの有意な上昇は認められなかったのに対して，プランルカスト群では有意な上昇が認められ，睡眠が障害されている可能性が示唆された（図8）。花粉飛散初期でスギ花粉症患者のPSQIのサブスケールを検討した結果，ベポタスチン群ではプランルカスト群で比較して入眠時間のスコ

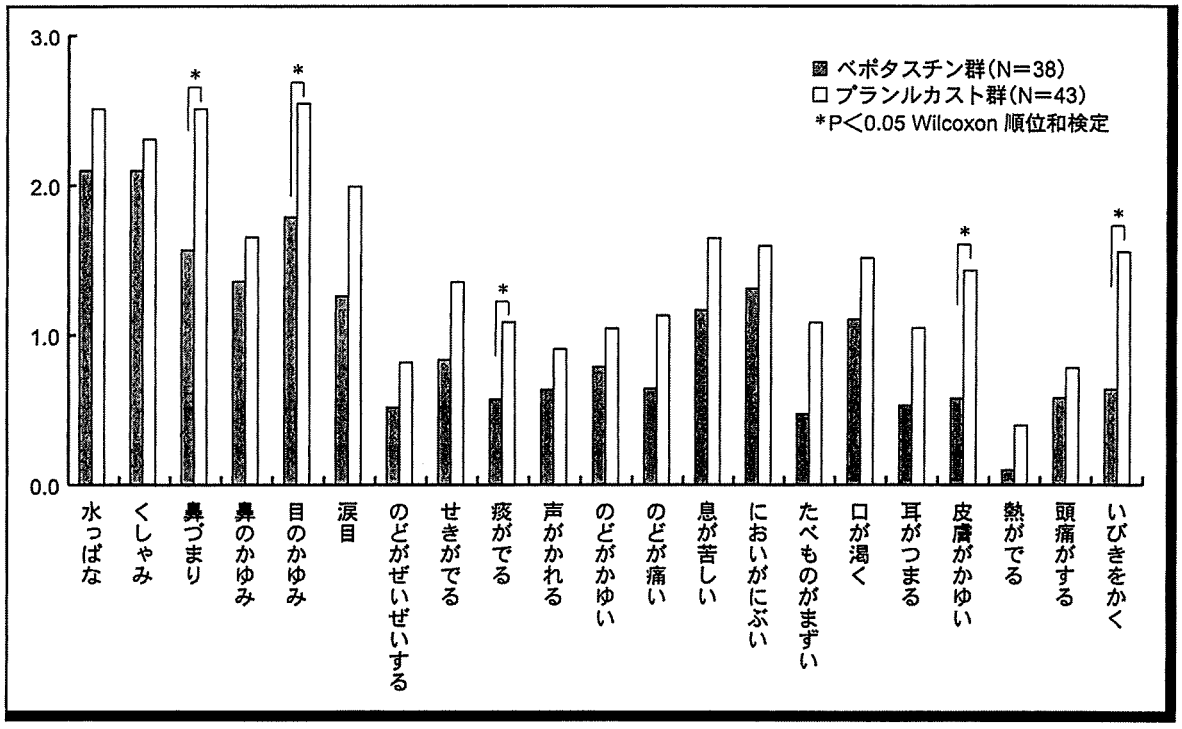

図7　JRQLQ (No.2) スギ花粉飛散ピーク時期の各項目

ベポタスチン群では，プランルカスト群と比較して痰，皮膚のかゆみ，いびきの各項目で有意なスコアの低下を認めた．

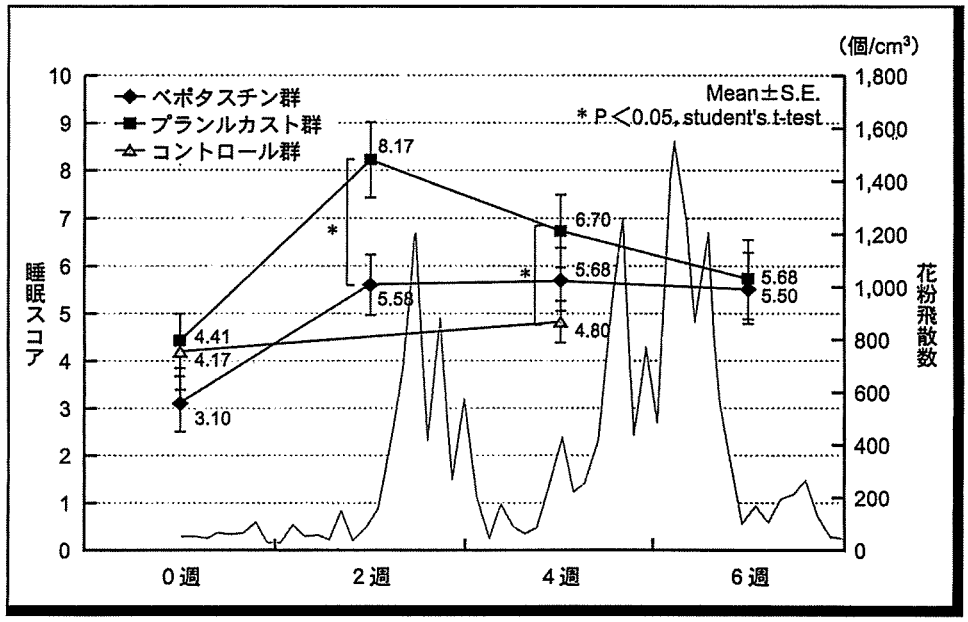

図8　2009 年度のスギ花粉飛散数と睡眠障害

ベポタスチン群では，プランルカスト群と比較してスギ花粉飛散２週目で睡眠障害スコアの有意な低下を認めた．

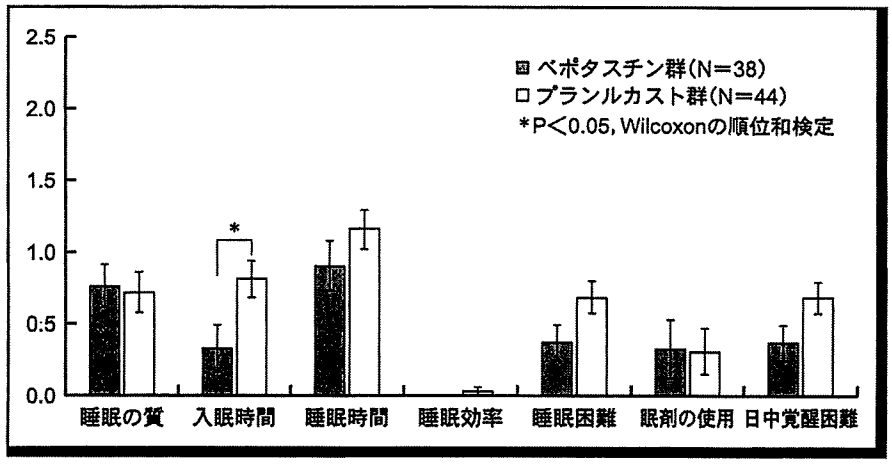

図9　スギ花粉飛散初期のPSQIの各項目
ベポタスチン群では，プランルカスト群と比較して入眠時間スコアの明らかな低下を認めた.

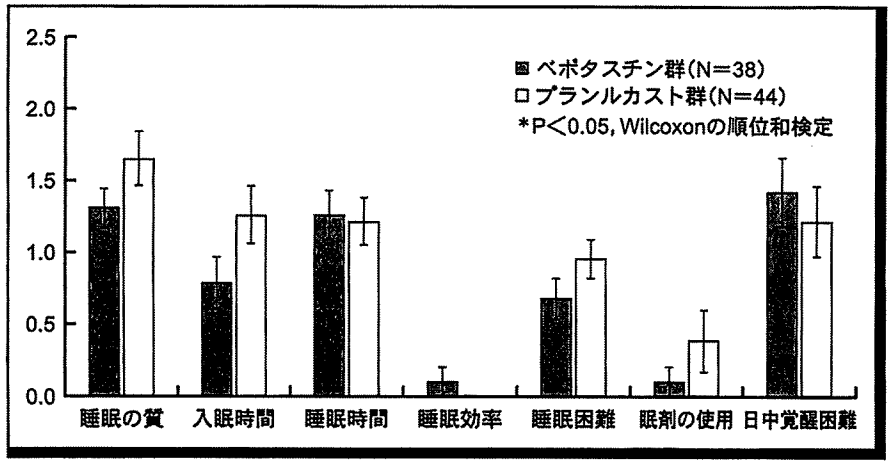

図10　スギ花粉飛散ピーク時のPSQIの各項目
両群でPSQIの各項目で有意な差を認めなかった.

アの有意な低下が認められた（図9，10）。

4．副作用

両群ともに問題となる副作用は認められなかった。

Ⅲ．考察

スギ花粉症では，健常人と比較してスギ花粉飛散数の増加に伴って鼻や眼の症状が増悪するだけでなくQOLも低下すると報告されている[3)~7)]。我々はスギ花粉症に対する初期治療の効果をJRQLQおよびPSQIを用いて検討した。スギ花粉飛散期の症状改善には第2世代抗ヒスタミン薬が広く用いられ，さらに点鼻薬や点眼薬などの局所外用薬も必要に応じて併用される。鼻アレルギー診療ガイドラインでは，第2世代抗ヒスタミン薬のアレルギー性鼻炎に対する推奨度は「A」であり，使用が最も強く推奨される薬剤と評価されている。また同ガイドラインでは，初期治療が推奨されておりケミカルメディエーター遊離抑制薬，第2世代抗ヒスタミン薬あるいはロイコトリエン

受容体拮抗薬などの花粉飛散開始早期からの投与によって，鼻粘膜の過敏性亢進が抑制され，スギ花粉症患者の症状発現時期を遅らせ，飛散ピーク時期の症状を軽減する効果があると考えられている[2]。本試験においては，本ガイドラインの推奨する初期投与薬剤であるロイコトリエン受容体拮抗薬と第2世代抗ヒスタミン薬の両者を用いて，大量飛散年における初期治療薬としての有用性について検討した。その結果，ベポタスチン群ではQOLがプランルカスト群と比較して有意に障害が軽度であった。また，睡眠障害も花粉飛散初期に有意に抑制されていた。アレルギー性鼻炎の睡眠障害に関する検討では，鼻症状の悪化が明らかに睡眠を障害し，特に鼻閉が大きく関与していると報告されている[12]~[18]。今回の検討では，鼻閉への有効性が高いロイコトリエン受容体拮抗薬がH_1受容体拮抗薬より睡眠障害を抑制すると予想されたが，2009年が大量飛散年で花粉飛散開始日から急激に飛散花粉数が増加し，症状が急激に悪化する状況では抗ヒスタミン作用のある薬剤の方の有用性が高かったものと推察される。スギ花粉症患者は若年層にも多く，労働や学習活動への影響が大きいと報告されている[17]。したがって，スギ花粉症の治療には眼・鼻の症状を制御することが重要であるだけでなく，QOLおよび睡眠障害など全身的な要因にも留意して治療戦略を考えることが肝要である。今回の検討から，ベポタスチンベシル酸塩はスギ花粉症の初期治療薬として優れた臨床効果を持ち，スギ花粉症の初期治療薬として有用である可能性が示唆された。

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分担研究報告書

鼻炎症状と喘息症状の連関についてのアンケート調査

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研究要旨
スギ花粉症における舌下ペプチド・アジュバンド療法開発の初期段階として、まずアレルゲン免疫療法を普及させ一般化させる必要がある。免疫療法の重要な恩恵として、いわゆるone airway one diseaseの抜本治療としての期待がある。アレルギー性鼻炎と気管支喘息は、互いに影響することがよく知られているが、特に我が国において、鼻疾患と喘息の連関に関する疫学的データは少ない。今回我々は、鼻疾患と喘息を合併する126名を対象に、鼻症状と喘息症状の連関の頻度及び特徴を調査する目的で、アンケート調査を行った。鼻疾患と喘息を合併する患者において、約30%で両者の連関を自覚していた。喘息コントロールのよくない患者では、鼻症状の悪化に伴い喘息症状が悪化しやすく、鼻治療により喘息症状が改善しやすいと自覚していることがわかった。従って鼻に対する治療は、通年的に喘息症状がある患者で特に重要であり、鼻症状と喘息症状のある患者に対しては、アレルゲン免疫療法を含む、上下気道にわたる包括的なアレルギー診療が必要と考えられた。

A. 研究目的

本研究班全体としてのテーマは、リアルタイムモニター花粉情報に加え、アレルゲン免疫療法の新規のアプローチである舌下ペプチド・アジュバンド療法の臨床研究である。舌下ペプチド療法を開発普及させる初期段階として、まずアレルゲン免疫療法を普及させ、一般化させることはきわめて重要である。免疫療法の重要な恩恵として、花粉症・アレルギー性鼻炎に高率に合併する喘息症状に対しても効果を発揮する、いわゆる one airway one disease の抜本治療としての期待がある。

アレルギー性鼻炎と気管支喘息が合併する割合は高く、アレルギー性鼻炎の 30-40%に気管支喘息が、気管支喘息の 50-80%にアレルギー性鼻炎が合併するとされている。アレルギー性鼻炎患者では、気管支喘息の有無と無関係に、気道過敏性亢進や好酸球の気管支への集積が見られる。また鼻粘膜へのアレルゲン曝露が、下気道の平滑筋収縮、好酸球浸潤、気道過敏性亢進を誘導することも報告されている。さらに、鼻炎の治療を行うことで、喘息症状や気道過敏性を改善させ、急性増悪頻度を減少させうることも報告されている。一方で、気管支喘息患者においても、鼻炎の有無と無関係に、好酸球の上気道への集積が見られる。また経気管支鏡的気管内アレルゲン投与が、鼻炎症状と鼻粘膜組織への好酸球浸潤を誘導することも報告されている。このように上気道と下気道は、共通の進行する炎症性反応の影響を受け、連動するメカニズム(one airway one disease)により持続・増幅すると考えられている。

しかしながら、鼻炎と喘息の合併例における上気道症状と下気道症状の連関は、日常臨床において、すべての患者で観察されるものではない。また、両者の連関がどのような患者で観察されやすいかについては、今まであまり検討されてこなかった。今回我々は、鼻炎症状と喘息症状の連関についてアンケート調査を行い、その連関の頻度および特徴について検討した。

B. 研究方法

埼玉医科大学病院アレルギー・喘息センター、あるいは耳鼻咽喉科に通院中の鼻炎症状と喘息症状を合併した患者 126 人を対象に、両者の連関についてアンケート調査を行った。性別は、男性 49 名、女性 75 名、記載なし 2 名であり、平均年齢は 48.1 歳であった。基礎疾患の内訳は、アレルギー性鼻炎 82 名、花粉症 74 名、慢性副鼻腔炎 28 名、気管支喘息 113 名、咳喘息 16 名であった。アンケート調査により、①鼻症状と喘息症状の連関の頻度②どのような患者で、鼻症状の変化によって、喘息症状が影響されるか、について検討した。統計学的解析として、異なる 2 群における検討には $\chi 2$ 乗検定を用いた。

C. 研究結果

鼻症状と喘息症状を合併した患者126名のうち、38名(30%)で、鼻症状の悪化に伴って喘息症状が悪化することを自覚し、28名(22%)で、鼻治療により喘息症状が改善することを自覚していた。一方、28名(22%)で、喘息症状の悪化に伴って鼻症状が悪化すると自覚し、24名(19%)で、喘息治療により鼻症状が改善することを自覚していた。

どのような患者で、鼻症状の変化によって、喘

息症状が影響されるか、についての解析は、アンケートの回答が得られた99名を対象とした。鼻症状の変化が喘息症状に与える影響は、喘息コントロールが良くない患者で有意に強かった。すなわち、通年的に喘息症状がある群では、喘息症状がない群と比較し、鼻炎症状の悪化により、喘息症状が悪化するとより自覚していることがわかった(喘息症状あり53%(31/58); 喘息症状なし17%(7/41); p=0.0002)。さらに、通年的に喘息症状のある群では、鼻症状の治療により喘息症状がより改善すると自覚していた(喘息症状あり36%(21/58); 喘息症状なし17%(7/41); p=0.03)。鼻炎症状の悪化に伴う喘息症状の悪化を自覚する頻度及び鼻炎治療に伴う喘息症状の改善を自覚する頻度は、喘息の重症度別で有意差はなかったが、最重症持続型（ステップ4でコントロール不良）に相当する5名においては、5名全員が、鼻炎症状が悪化すると喘息症状が悪化すると回答し、重症度が高くなると両者の連関を認識しやすくなる可能性が示唆された。また、副鼻腔炎のある患者で両者の連関はより強い傾向にあった。

D. 考察

鼻疾患と喘息を合併する患者において、約30%で両者の連関を自覚していた。鼻に対する治療は、通年的に喘息症状がある患者で特に、喘息コントロールに重要と考えられた。鼻炎症状に伴って喘息症状が悪化する機序としては、①アレルゲンが直接下気道に到達し喘息症状を誘発する②鼻閉塞によってアレルゲンが下気道に侵入しやすくなる③鼻局所でロイコトリエンなどの化学伝達物質が産生・放出され、一部が下気道に下降する④アレルゲン曝露に伴いIL-5などのTh2サイトカインの産生が亢進し、骨髄に作用し好酸球を増加または活性化させ、気道への好酸球浸潤が増加する、などが想定されている。実際には、これらが複合的に病態形成に関与していると考えられるが、それぞれの関与の割合は不明である。自覚症状から見た場合、普段から喘息コントロールを良くすることが、鼻炎症状と喘息症状を合併している患者の管理において特に重要と考えられた。

E. 結論

鼻疾患と喘息を合併する患者において、約30%で両者の連関を自覚していることを確認した。通年的に喘息症状がある患者では、鼻治療により喘息症状が改善すると自覚しており、鼻治療は喘息コントロールに重要と考えられた。鼻症状と喘息症状のある患者に対しては、舌下ペプチド・アジュバンド療法を含むアレルゲン免疫療法などの、上下気道にわたる包括的なアレルギー診療が必要と考えられた。

F. 健康危険情報

該当事項なし

G. 研究発表

1. 論文発表
   該当事項なし
2. 学会発表
   該当事項なし

H. 知的財産権の出願・登録状況(予定を含む)

1. 特許取得
   該当事項なし
2. 実用新案登録
   該当事項なし
3. その他
   該当事項なし

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分担研究報告書

スギ花粉症治療に関する遺伝子解析からの検討

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研究要旨：
我々の最新疫学調査においてアレルギー性鼻炎の罹患率及び抗原感作率はかなり増加していることが判明した。その原因はほとんど解明されていないので、遺伝子的側面からのアプローチを行った。スギ花粉症と陰性コントロールの2群において候補遺伝子アプローチと全ゲノム解析を行った。その結果、候補遺伝子アプローチでは、IFNγR 1 プロモーター領域(rs2234711)において、リスクアレルはCであり(p=0.030)、危険率は1.24を示した。リスクアレルのIFNγRプロモーター領域への組み込みによるプロモーターアッセイでは、リスクアレルにおいてIFNγRの発現が高いことが示唆された。IL-33はrs1929992においてリスクアレルがCであった(p<0.05)。血清中IL-33は、スギ花粉症においてコントロール、副鼻腔炎に対して有意に高値を示した(p=0.0018)。全ゲノム解析では、三次解析にて Decay-accelerating factor (DAF) rs10746463においてリスクアレルAであった(p=0.00033)。rs10746463がA/Aになっているとと DAF 蛋白の発現が低く、血清中総IgEとスギ特異的IgEの両者とも高値であった。今回の研究でスギ花粉症治療における3つの標的分子が同定できた。

A. 研究目的

2006年～2008年に当教室で行った20歳から50歳まで約2000名の疫学調査で、7項目の吸入アレルゲン(ヤケヒョウヒダニ、コナヒョウヒダニ、スギ、カモガヤ、ブタクサ、カンジダ、アスペルギルス)のうち1項目でも抗原特異的IgEが陽性である人は、70%を占めることが判明した。またこの10年間でスギ花粉症の発症率も10%以上増加したことも判明した。その原因のいくらかは、遺伝的要因も占めると思われる。そこで今回は、候補遺伝子アプローチと全ゲノム解析による遺伝子多型(SNP)の二つの方法にて、スギ花粉症患者の特徴を検討した。得られた遺伝子情報を元に、スギ花粉症治療に関する新規標的分子を開発する目的のためこの研究を行った。

B. 研究方法

候補遺伝子アプローチは、研究対象者として、スギ花粉症患者619名、吸入アレルゲン7項目がいずれも陰性であり、アレルギー疾患の既往ないもの(陰性コントロール)311名を用いた。候補遺伝子としてサイトカイン、ケモカイン、

それらの受容体、TLRを含む自然免疫関連、シグナル伝達物質、制御性T細胞関連、アポトーシス関連、ウイルス感染関連など約100項目の遺伝子、各遺伝子において約5から10のSNPを調べた。有意差を認めた遺伝子に関しては、プロモーターアッセイを用いて遺伝子多型部位の機能解析を行った。

全ゲノム解析では、一次解析として10万SNPをスギ花粉症93例とアレルギー陰性コントロール45例、二次解析としてスギ花粉症234例とアレルギー陰性コントロール150例、3次解析としてスギ花粉症370例とアレルギー陰性コントロール235例で検討した。

C. 研究結果

候補遺伝子アプローチにて有意差を認めたのは、Th1 サイトカインの代表である IFNγの受容体(IFNγR)と IL-1 と関連深く IL-1 受容体のリガンドであり、Th2 反応に重要な IL-33 の二つであった。

IFNγR において IFNγR 1 プロモーター領域(rs2234711)の SNP とスギ花粉症が相関を認めた。スギ花粉症でのリスクアレルは、Cであり(p=0.030)、危険率は1.24であった。この領域は成人アトピー型喘息での SNP と同様であった。このリスクアレルを IFNγR 1 プロモーター領域のベクターに組み込み、ヒト単球系細胞株 THP-1 に遺伝子導入し、IFNγ(30ng/ml)添加して3時間培養した後、ルシフェラーゼアッセイを行うとリスクアレルCにおいてIFNγRの発現が高いことが示唆された。

IL-33においてはrs1929992においてリスクアレルがCであった（$p<0.05$）。血清中IL-33は、スギ花粉症において陰性コントロール、および感染性鼻炎に比較して、有意に高値を示した（$p=0.0018$）。

全ゲノム解析では、一次解析にて384 SNPに絞られ、二次解析にて26 SNPに絞られた。その中でDecay-accelerating factor (DAF)に注目し解析を行ったところ、rs10746463においてリスクアレルAがスギ花粉症と有意な相関を認めた（$p=0.00033$）。またプラスミドベクターによるリスクハプロタイプを構築し、ルシフェラーゼアッセイを行ったところDAFの転写が有意に低下した。またDAFのプロモーター領域rs10746463がA/ Aになっていると血清中IgEの高値を認めた。IgEは総IgEとスギ特異的IgEの両方とも高値であった。これは、rs10746463がA/ Aになっていると DAF 蛋白の発現が低いことによることが判明した。

D. 考察

スギ花粉症において、成人アトピー型喘息と同じIFNγR 1プロモーター領域のSNPと相関が認められたことは、One airway, one diseaseの概念から、予想されたことであった。しかしリスクアレルの導入によりIFNγRの発現が高くなったことは、意外であった。一般的な考え方からするとリスクアレルの導入によって、IFNγRの発現が低くなり、IFNγの作用が影響しにくくなった時に、スギ花粉症や成人アトピー型喘息が発症すると考えた方が簡単であった。今回は、IFNγRの蛋白発現を検討したのみにすぎず、リスクアレルを含むIFNγRの機能解析（通常のIFNγRの働きをするのかという検討）は行っていないため、今後の検討を要する。もしIFNγRの機能上問題がなければ、今回の結果は、IFNγを介したアレルギー炎症の増悪、もしくはIFNγの減少によって作用を少しでも受けようとする受容体の増加とみるべきであろう。

IL-33は、Toll-インターロイキン1（IL-1）受容体（TIR）ドメイン含有受容体ST2と結合し、Th2免疫調節活性を有するとともに、Toll様受容体（TLR）の活性を阻害するとされる。そのため、IL-1のような因子の活性を阻害し、炎症を軽減する可能性がある。これまでIL-33受容体(ST2)のSNPが気管支喘息と関連や気管支喘息の症状とST2の変化が報告されてきた。スギ花粉症では、IL-33は高値を示し、まだその機能は十分にわからないが、何らかの役割を担っていることは確かである。

DAFは、補体系C3が活性化しC3aとなり、リンパ球、平滑筋、血管内皮細胞を活性するのを抑制する働きがあり、我々はスギ花粉症患者では、スギ花粉飛散期に、DAFの発現が低下するデータも別途得ている。すなわちDAF発現低下によって局所IgEが上昇し、アレルギー反応を増大させる可能がある。以上のことはDAFを強発現させるとアレルギー反応を抑制できるかもしれない。

E. 結論

スギ花粉症の遺伝子としてIFNγR 1プロモーター領域（rs2234711）、IL-33（rs1929992）、DAF（rs10746463）が同定できた。機能解析からIFNγRの蛋白発現亢進、スギ花粉症患者におけるIL-33の高値、DAF発現低下に伴うIgE上昇が認められ、それぞれが今後スギ花粉症の治療に結び付く可能性があると思われた。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む）

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

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ORIGINAL ARTICLE **Asthma and Rhinitis**

Associations between decay-accelerating factor polymorphisms and allergic respiratory diseases

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Clinical & Experimental Allergy

Summary

Background Allergic diseases such as asthma and allergic rhinitis are major causes of morbidity in developed countries. The pathology underlying allergic respiratory diseases is considered to be IgE-mediated type I allergy characterized by mucosal inflammation that occurs in response to allergen exposure. They are common diseases involving a complex inheritance. Complement systems are known to play an important role in allergic diseases. Decay-accelerating factor (DAF) is important for the regulation of the complement system and is a good candidate for determining the susceptibility to allergic diseases.

Objective The present study aimed to investigate whether polymorphisms in the *DAF* gene are associated with allergic respiratory diseases in the Japanese population.

Methods We performed mutation screenings of DAF and conducted a tag single-nucleotide polymorphisms (SNP) association analysis for 684 unrelated adult individuals with seasonal allergic rhinitis (SAR) with Japanese ceder pollen, 188 mite-sensitive adults with asthma, and 346 unrelated non-allergic healthy controls.

Results DAF is located in the tight linkage disequilibrium (LD) block spanning 62 kb. The tag SNP analysis revealed that rs10746463 was significantly associated with SAR ($P = 0.00033$) and mite-sensitive adult asthma ($P = 0.044$). The rs2564978 and rs3841376 haplotypes, which are located in the promoter region of *DAF*, were in complete LD with rs10746463 ($r^2 = 1$). Luciferase reporter assays with constructs containing the 5′ flanking regions of *DAF* showed that the plasmid with rs2564978 C/rs3841376 deletion (the risk haplotype) had a statistically significantly lower transcriptional activity than that containing the rs2564978 T/rs3841376 insertion.

Conclusions Our results suggest that *DAF* is one of the genes involved in conferring susceptibility to allergic respiratory diseases and show that decreased levels of *DAF* may be associated with the enhanced specific IgE responses occurring in allergic diseases in the Japanese population.

Keywords Japanese cedar, luciferase assay, polymorphism

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Introduction

Allergic diseases such as asthma and allergic rhinitis are major causes of morbidity in developed countries, and their incidence is increasing. Seasonal allergic rhinitis (SAR) by Japanese cedar (*Cryptomeria japonica*; JC) is an IgE-mediated type I allergy affecting the nasal mucosa. It occurs after an exposure to JC pollen. It is one of the most common allergic diseases in Japan, affecting 19.4% of the Japanese population [1]. It is thus a major public health issue in Japan. According to a national survey, the prevalence of rhinitis in Japan was 0.16 in 1992 and 0.21 in 2002 [2]. Over 16% of the Japanese population suffers from the allergy during JC seasons. SAR therefore contributes to the undermined quality of life and decline in labour productivity [3].

Epidemiologic studies have consistently shown that asthma and rhinitis often coexist in the same patients in

every region of the world [4], suggesting the concept of 'one airway, one disease' [5]. Currently, approximately 300 million people worldwide have asthma, and this disease claims the lives of 180 000 people every year [6]. Increased levels of IgE against common environmental allergens are considered as the strongest predisposing factor for asthma, and dust mite allergy is strongly associated with asthma [7].

It is generally accepted that allergic diseases such as asthma and rhinitis are the result of inappropriate immunological responses to common environmental allergens in genetically susceptible individuals [8]. Twin and family studies have confirmed the existence of a genetic predisposition to the development of asthma and allergic rhinitis, with a heritability of 0.71 and 0.69, respectively [9], although a clear Mendelian pattern of inheritance has not been established.

It is well known that patients with allergic rhinitis and asthma develop T helper type 2 (Th2) polarized immune responses and high IgE responsiveness against allergens. However, the important role of other immune systems such as the complement system in allergic inflammation has also been demonstrated [10, 11]. Genetic studies have reported the association of polymorphisms in complement 3 (C3) with asthma in various populations [12, 13]. Activation of the complement system produces proteins called anaphylatoxins, which are the cleavage products of C3 (C3a) and C5 (C5a). Anaphylatoxins have a variety of effects on cells: they cause smooth muscle contraction, enhance vascular permeability, and act as chemotactants for a wide variety of leucocytes [11]. Thus, inappropriate regulation of the complement system may damage the host tissues [14]. A number of membrane-bound proteins such as the decay-accelerating factor (DAF) play an important role in the regulation of the complement system. The physiological role of DAF is to inhibit the complement cascade at the critical C3 convertase stage by binding to the C3 and C5 convertases, and consequently accelerating the decay of the enzyme subunit [15]. In this study, to elucidate the role of DAF in the development of allergic respiratory diseases, we performed a single-nucleotide polymorphism (SNP) association study with unrelated patients having JC-induced SAR or mite-sensitive asthma, and unrelated Japanese non-allergic healthy controls.

Materials and methods

Subjects

Patients with JC-induced SAR were recruited from among patients who visited the otolaryngology department of the University Hospital of Fukui, Japan, and from among hospital workers and students of the same university hospital. Healthy, non-allergic controls were also recruited from among the hospital workers and students of the University Hospital of Fukui. The recruitment was carried out between 2005 and 2007; all the subjects were residents of Fukui City, Japan. The diagnosis of JC-induced SAR was based on a positive history of rhinitis during the cedar pollen season and high levels of allergen-specific IgE antibodies in the serum (RAST score $\geqslant$class 2); the diagnosis was confirmed by the participating otolaryngologists. The control group comprised healthy adult subjects without any allergic symptoms and with no allergen-specific IgE antibodies against common inhaled allergens [JC pollen, orchard grass, and house dust mite (HDM)].

We recruited 188 adult atopic asthma patients sensitive to HDM (*Dermatophagoides farina* or *Dermatophagoides pteronyssinus* RAST score $\geqslant$class 2) from Miyatake asthma clinic (mean age, 46.8±14.8 years; male : female ratio, 0.97).

All participants gave their written informed consent to participate in the study. The re-sequencing panel consisted of 32 SAR patients. This study was approved by the Ethical Committee of University of Tsukuba and University of Fukui, Japan.

Tag single-nucleotide polymorphisms analysis of decay-accelerating factor

The genotype data of the *DAF* region of an Asian population (Japanese from Tokyo, Japan, and Han Chinese from Beijing, China) were downloaded from the HapMap website (http://www.hapmap.org/, data release #21), and tag SNPs were selected using the Tagger software [16] implemented in the Haploview software [17], with an r^2 threshold of 0.8 and allele frequencies of 0.1. In 32 patients, we performed re-sequencing of the 5′ flanking region in *DAF* using the primer pair 5′-ATTGTTATCC CACCCCACAC-3′ and 5′-GACAAACAAGACGGGTGGA-3′ that amplified the region located from −633 to +126 bp relative to the transcription initiation site. Next, we genotyped tag SNPs as well as the newly identified SNPs that were not in a strong linkage disequilibrium (LD) with other typed SNPs. The SNPs were genotyped using the TaqMan Assay-on-Demand™ SNP Typing Systems (Applied Biosystems, Foster City, CA, USA) by following the manufacturer's instructions.

Reporter assay

We generated luciferase reporter gene constructs containing the 5′ upstream region of *DAF* spanning from position −441 to +289 bp relative to the transcription initiation site; this region contained two polymorphisms that were in complete LD ($r^2 = 1$): the rs2564978 T/C polymorphism was located at −401 bp relative to the transcription initiation site and the 21 bp insertion/deletion polymorphism rs3841376 was located at −333 bp relative to the

transcription initiation site. This region was reported to contain fragments responsible for the transcriptional activity [18]. Finally, two constructs were generated: one with rs2564978T/rs3841376 insertion and the other with rs2564978 C/rs3841376 deletion. The fragments were amplified using the primers 5′-GACTGCTAGCCGAACAAGG CATGAACAA-3′ and 5′-GTCAAAGCTTGCCGGGTTAGAA CAAGGA-3′. The products were digested with *Nhe*I and *Xho*I (New England Biolabs, Beverly, MA, USA) overnight at 37 °C and then subcloned into *Nhe*I- and *Hind* III-digested pGL3-Basic Vector (Promega, Madison, WI, USA). The orientation and accuracy of the inserted fragments were confirmed by direct sequencing. A549 cells (human lung adenocarcinoma epithelial cell line; RIKEN Bio-Resource Center, Tsukuba, Ibaraki, Japan) were cultured in Eagle's minimum essential medium (Sigma-Aldrich Co., St. Louis, MO, USA) supplemented with 10% fetal calf serum (Sigma-Aldrich Co.) at 37 °C with 5% CO_2. Approximately 2×10^6 cells were cotransfected with 1 μg of the test construct and 100 ng of pRL-TK (Promega) supplemented with HilyMax (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions. The cells were incubated at 37 °C for 48 h and later disrupted by adding 500 μL of lysis buffer (Promega). Twenty microlitres of each lysate was used for the luciferase assay, which was performed using the Dual-luciferase reporter assay system (Promega). The expression efficiency was measured using a TD 20/20 luminometer (Turner Designs Instruments, Sunnyvale, CA, USA). The firefly luciferase values were normalized to the Renilla luciferase values of pRL-TK, which were determined simultaneously. Reporter activity is presented as the mean of three independent values.

Statistical analysis

Any deviation from the predicted Hardy–Weinberg frequencies and the significance of the differences in the case–control samples (proportion of gender) were determined using a χ^2 test. Continuous variables such as age, total, and specific IgE were compared with Student's *t*-test. Statistical evaluations for testing the genetic effects of the association between the case–control status and each individual SNP were estimated by logistic regression analysis after adjusting for gender and age. Association analyses were performed assuming an additive, dominant, and recessive effect for each polymorphism using SNPassoc software [19]. Haplotype analysis was performed using the additive model with the Haplo.stats software version 1.1.0 [20]. We also performed linear regression analysis to examine the effect of these variants on the quantitative variables of total serum IgE and specific IgE against JC pollen. Multiple comparisons were corrected by Bonferroni corrections, and corrected *P*-values of <0.05 were considered significant.

The differences in transcriptional activity, which were determined by the luciferase assay, were analysed using Student's *t*-test. The expression levels of *DAF* in the Epstein–Barr virus-transformed lymphoblastoid cell lines derived from Chinese and Japanese populations were retrieved from the GENe Expression VARiation database (GENEVAR) [21]. The differences in *DAF* gene expression in each genotype were analysed using Student's *t*-test.

Results

The clinical details of the patients with JC-induced SAR, those with mite-sensitive asthma, and controls are listed in Table 1. Significant differences in the age, sex ratio, and other than the total and specific IgE levels were observed between cases and controls. Therefore, we performed logistic regression analysis after adjusting for gender and age. The LD map of the *DAF* gene region is shown in Fig. 1. *DAF* is located in the tight LD block spanning 62 kb between rs6686201 and rs2782837. Three tag SNPs (rs6691942, rs10746463, and rs2782837) were genotyped, and 1 SNP – rs10746463 – was found to be associated with JC-induced SAR (Table 2). The genotype frequencies of these three SNPs did not show a deviation from the Hardy–Weinberg equilibrium ($P>0.05$).

Next, we tested the association of rs10746463 with the SAR-related quantitative phenotypes (total serum IgE and JC-specific IgE) using linear regression analysis after adjusting for the effects of age and gender. The additive effects of the rs10746463 A allele for total serum IgE and JC-specific IgE were observed, but this effect disappeared after adjusting for the affection status (Table 3). The results of the haplotype analysis conducted after adjusting for age and gender are shown in Table 4. The individual SNP as well as haplotype analyses revealed a significant association of the *DAF* variants with the development of SAR.

In order to evaluate the effects of the *DAF* variant on the development of other allergic diseases, we performed an association analysis with patients with HDM-sensitive adult atopic asthma. Logistic regression analysis performed after adjusting for gender and age revealed that rs10746463 was also associated with mite-sensitive asthma (Table 5; $P=0.043$; recessive model).

The re-sequencing of the *DAF* promoter region identified 3 SNPs (rs2564978, rs3841376, and rs28371583) in 32 individuals (Fig. 2). Among them, rs2564978 and rs3841376 were in complete LD with rs10746463 ($r^2=1$). The haplotypes of rs3841376, rs2564978, and rs10746463 were rs2564978 C/rs3841376 deletion/rs10746463 A (the risk haplotype for allergic respiratory diseases) and rs2564978 T/rs3841376 insertion/rs10746463 G. The SNP rs3841376 is a 21-bp insertion/deletion polymorphism located 333 bp upstream of the transcription initiation site. Therefore, it is possible that rs3841376 can influence

Table 1. Characteristics of patients and controls

	JC-induced SAR ($n=684$)	Mite-sensitive asthmatics ($n=188$)	Controls ($n=346$)
Age	33.0+9.6*	46.8±14.8*	34.5+9.8
Male/female ratio	0.36*	0.97*	0.53
IgE (IU/mL, mean, range)	469 (10–11 000)*	688 (10–8710)*	35 (0–330)
Japanese ceder pollen-specific IgE (UA/mL, mean, range)	30.9 (0.71–> 100)*	NA	<0.34
Dermatophagoides pteronyssinus-specific IgE (UA/mL, mean, range)	8.9 (<0.34–> 100)*	24.4 (0.71–> 100)*	<0.34
Dermatophagoides farina-specific IgE (UA/mL, mean, range)	12.0 (<0.34–> 100)*	23.5 (0.71–> 100)*	<0.34

*$P<0.05$ between cases and controls.
NA, data not available; JC, Japanese cedar; SAR, seasonal allergic rhinitis.

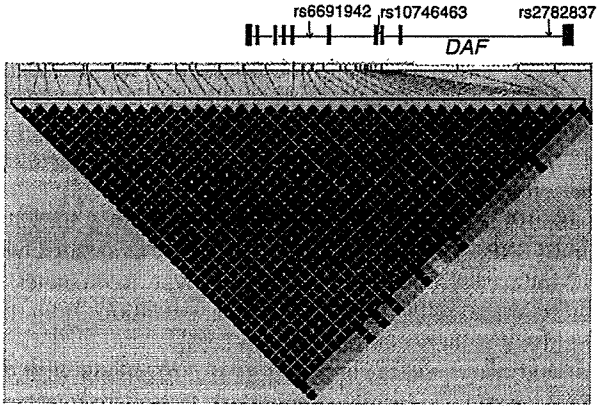

Fig. 1. Pairwise linkage disequilibrium between decay-accelerating factor (*DAF*) polymorphisms in a 62-kb region as measured by r^2 in Asian HapMap subjects. The r^2 values for linkage disequilibrium (LD) are colour-coded by Haploview software, and the extent of red indicates the strength of LD. The positions of the single-nucleotide polymorphisms are indicated with arrows.

the transcriptional activity of the *DAF* gene. We constructed plasmids that contained the 5′ upstream region of *DAF*; this region contained the rs2564978 and rs3841376 polymorphic sites. The plasmid containing the rs2564978 C/rs3841376 deletion (the risk haplotype for allergic respiratory diseases) showed a statistically significantly lower transcriptional activity than that containing rs2564978 T/rs3841376 insertion (Fig. 3, $P=0.02$). The Sanger Institute GENEVAR expression database [21] shows a strong association between the SNP rs10746463 A, which was in complete LD with the rs2564978 C/rs3841376 deletion haplotype, and the differences in the *DAF* expression levels. Further, the expression levels of *DAF* in the AA ($n=17$) genotype were found to be lower than that of AG ($n=53$) and GG ($n=19$) genotypes ($P<0.05$, Fig. 4).

Discussion

Our results indicate that the *DAF* SNP is associated with the development of both JC-induced SAR and mite-sensitive asthma. The luciferase transcription assay suggested that the A allele of rs10746463, which is associated with an increased susceptibility to respiratory allergic diseases, decreases the transcriptional activity of *DAF*; this finding is concordant with the pattern of immortalized B cell expression. To our knowledge, this is the first study to show that the *DAF* genotypes and haplotypes were associated with allergic diseases in humans, and our results suggest that decreased levels of *DAF* may be associated with enhanced specific-IgE responses in allergic diseases.

DAF, which is also known as CD55 and is produced by a variety of cells, regulates the complement system by accelerating the decay of the enzyme subunit. It is involved in the pathogenesis of various diseases, including paroxysmal nocturnal haemoglobinuria [22], autoimmune diseases [23], and cancer [24]. DAF also acts as the receptor for pathogens such as the echovirus [25] and *Helicobacter pylori* [26].

Complements play an important role in the innate and adaptive immunity. The complement system has the potential to cause extreme damage to the host tissues; therefore, its activation is tightly regulated by complement regulatory proteins such as DAF, CD46, and CD59. DAF acts by binding to the C3 and C5 convertases of all complement activation pathways. It regulates complement activation at the critical C3 convertase stage by preventing the assembly of C3 convertase and by accelerating the decay of preformed C3 convertases. Therefore, DAF prevents the formation of the anaphylactic cleavage fragments C3a and C5a. It has also been reported that complement activation products such as C3a and C5a contribute to the inflammation of allergic rhinitis. Andersson et al. [27] showed that an allergen challenge test administered to allergic subjects induced nasal symptoms and concomitantly increased the C3a and C5a levels. These levels were also increased in the bronchoalveolar lavage fluid collected after segmental allergen provocation in subjects with allergic asthma [28]. A recent study has reported that the level of C3a receptor expression was significantly higher in the nasal mucosa samples of patients with severe persistent allergy than in the nasal

Table 2. Association of DAF SNPs with SAR

SNP	Location*	Genotype count frequency				Additive P (corrected)†	Dominant P (corrected)†	Recessive P (corrected)†
rs28371583	205561302	Case	534 (0.78)	139 (0.20)	11 (0.02)	0.04	0.052	0.27
A>G		Control	284 (0.82)	57 (0.17)	4 (0.01)	(0.48)	(0.62)	(1)
			CC	CT	TT			
rs6691942	205568150	Case	187 (0.28)	338 (0.50)	148 (0.22)	0.87	0.6	0.77
C>T		Control	103 (0.30)	163 (0.48)	77 (0.22)	(1)	(1)	(1)
			AA	AG	GG			
rs10746463	205577219	Case	223 (0.35)	327 (0.51)	90 (0.14)	0.0018	0.096	0.00033
A>G		Control	99 (0.29)	161 (0.47)	81 (0.24)	(0.022)	(1)	(0.004)
			CC	CT	TT			
rs2782837	205597549	Case	469 (0.69)	192 (0.28)	14 (0.02)	0.42	0.94	0.027
C>T		Control	240 (0.69)	89 (0.26)	17 (0.05)	(1)	(1)	(0.32)

*Locations are relative to the contig NT_021877.

†Corrected *P*-values by Bonferroni's correction.

DAF, decay-accelerating factor; SAR, seasonal allergic rhinitis; SNP, single-nucleotide polymorphisms.

Table 3. Association analysis between rs10746463 and total serum IgE and between rs10746463 and JC-specific IgE

Genotype	Mean log (total serum IgE)+SE	*P*-values (*P* adjusted by affection status)
AA	199+0.036	
AG	1.88+0.03	0.006 (0.40)
GG	1.73+0.045	
Mean log (JC-specific IgE)+SE		
AA	0.65+0.05	
AG	0.62+0.041	0.0011 (0.53)
GG	0.35+0.68	

JC, Japanese cedar.

Table 4. Haplotype association test

Haplotype	Haplotype frequency	*P*-value (corrected)
ATAC	0.44	0.058 (0.41)
ACGC	0.24	0.27 (1)
ACGT	0.15	0.8 (1)
GCAC	0.1	0.0066 (0.046)
ACAC	0.021	0.1 (0.7)
ATGC	0.02	0.000006 (0.000042)
ACAT	0.013	0.8 (1)

The SNP order in haplotypes is rs28371583, rs6691942, rs10746463 and rs2782837.

SNP, single-nucleotide polymorphisms.

Table 5. Association of the *DAF* SNP with mite-sensitive asthma

SNP	Genotype count (frequency)			Recessive model P
rs10746463	Case 60 (0.32)	99 (0.53)	29 (0.15)	0.044
A>G	Control 99 (0.29)	161 (0.47)	81 (0.24)	

SNP, single-nucleotide polymorphisms; *DAF*, decay-accelerating factor.

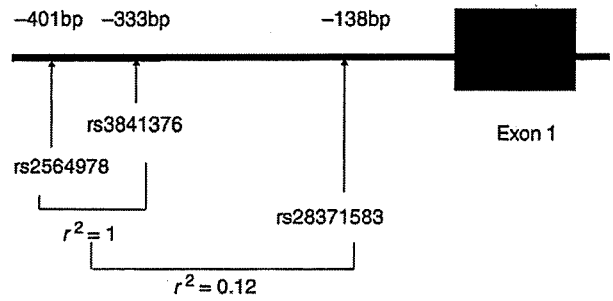

Fig. 2. Locations of the single-nucleotide polymorphisms in the 5′ promoter region of decay-accelerating factor (*DAF*). The distances are relative to the transcription initiation site.

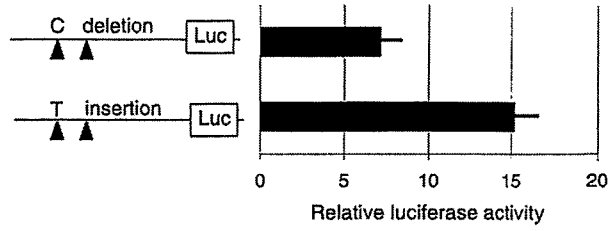

Fig. 3. Comparison of polymorphisms of decay-accelerating factor (*DAF*) analysed by relative luciferase activities in A549 cells. Results are expressed as relative luciferase activity normalized to those of *Renilla* luciferase (pRL-TK). Standard deviation (SD) is indicated by the error bar. The mean relative activities and ±SD were calculated in three independent transfections. Statistical analysis was performed with Student's *t*-test.

mucosa samples of normal subjects and of patients with mild allergy [29]. This suggests that the C3a receptor may mediate mucus secretion and mucosal swelling in the allergic nasal mucosa, especially in cases of severe persistent allergy [29]. Our present data gathered from the Japanese population suggest that subjects possessing

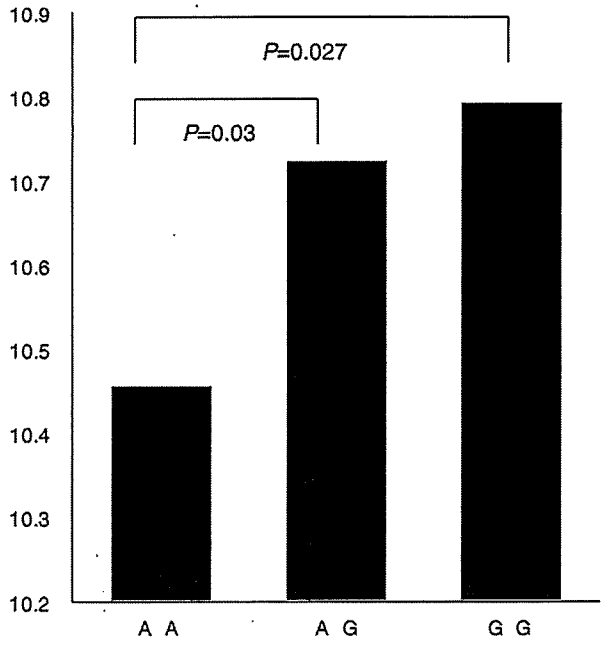

Fig. 4. Gene expression levels of Epstein–Barr virus-transformed lymphoblastoid cell lines from Chinese and Japanese populations according to their genotypes. Normalized expression value of each individual was retrieved from GENEVAR web site [21], and statistical analyses were performed with Student's *t*-test. The bars represent the means of the expression levels of decay-accelerating factor (*DAF*) in the subjects with AA ($n = 17$), AG ($n = 53$), and GG ($n = 19$) genotypes.

genotypes that down-regulate the transcriptional activity of *DAF* were more susceptible to allergic respiratory diseases. Hence, it is likely that decreased levels of DAF contribute to the production of C3a anaphylatoxins, resulting in the heightened susceptibility to allergic respiratory diseases.

Our results indicate that the *DAF* SNP is associated with the development of both JC-induced SAR and mite-sensitive asthma. It should be noted that our control subjects were super-controls, i.e. subjects not reactive to the common inhaled allergens. Therefore, our results can also imply that the rs10746463 GG has a protective effect against allergic diseases.

In summary, our study shows that a genetic variation in *DAF* significantly alters the susceptibility of an individual to allergic respiratory diseases. Our findings further support the role of the complement system in allergic diseases. Thus, our results would facilitate the understanding of the pathogenesis of allergic diseases and be valuable in research for novel treatments.

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Prevalence of Allergic Rhinitis and Sensitization to Common Aeroallergens in a Japanese Population

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Key Words
Aeroallergen · Allergic rhinitis · Dust mite · Specific human IgE · Japanese cedar pollen

Abstract
Background: Allergic rhinitis (AR) is recognized as a major health problem worldwide, and its prevalence depends on the age range of the subjects. The aims of this study were to determine the current prevalence of AR, effects of age on the prevalence of IgE sensitization to inhalant allergens, and serum total IgE levels in Japanese subjects. ***Methods:*** We conducted a survey of 1,540 subjects between 20 and 49 years of age in 2006 and 2007 and examined the prevalence of AR and sensitization to 7 common aeroallergens. We measured serum total IgE and specific IgE to 7 aeroallergens. AR was determined based on symptoms, predominantly in the nose and eyes, caused by aeroallergens as mentioned in a questionnaire and sensitization to any of the 7 aeroallergens as assessed by measurement of serum specific IgE. ***Results:*** The prevalence of AR was 44.2% (681 of the 1,540 subjects) and there was no difference among age decades. Of the 1,540 subjects, 1,073 (69.7%) were sensitized to at least 1 of the 7 aeroallergens. The most common allergen in AR was Japanese cedar pollen (89.6%, 610 of the 681 with AR) in all the age decades examined. The sensitization rate to mites was significantly higher in the younger subjects. ***Conclusion:*** Our data suggest that the prevalence of AR between 20 and 49 years of age has increased by nearly 10% during the last 10 years. Cedar pollen and mites were predominant allergen sources among the 7 aeroallergens in the Japanese population.

Introduction

Allergic rhinitis (AR), the most common type of rhinitis, is a heterogeneous disorder that significantly impairs the patient's quality of life, and its prevalence has markedly increased in recent decades [1, 2]. Epidemiologic and serological studies have provided valuable information to develop effective strategies for the prevention and treatment of the disease [3–6]. Japanese cedar

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pollinosis (JCP) is a common allergic disease, and the increase in its prevalence is a major public health problem in Japan [7]. Several epidemiologic studies have been conducted on JCP [8–11]. Sakurai et al. [9] reported the prevalence and risk factors of AR and JCP among 2,307 Japanese men; the prevalence rates of AR, seasonal rhinitis and JCP were 35.5, 28.8 and 11.0%, respectively, in 1998. Kaneko et al. [10] conducted a meta-regression analysis of 38 population-based surveys in Japan. The prevalence of JCP among adolescents in the general population was estimated at 28.7% in metropolitan areas and 24.5% in urban areas in the year 2004. The study also reported that the prevalence of JCP increased 2.6-fold between 1980 and 2000. To monitor the prevalence of sensitization is useful for understanding AR and developing preventive measures.

In AR, an IgE-mediated response to allergens is triggered and characterized by type-2-helper-T-cell-dependent inflammation [12]. Allergen-specific IgE is a critical factor in the mechanism of AR. Serum allergen-specific IgE results closely correlate to those of skin tests and nasal challenges. Allergen-specific IgE tests are highly specific and sensitive. One of their advantages is that drugs and skin diseases do not influence the measurement [1].

Sensitization is an important risk factor for developing allergic disease [13]. Epidemiological investigation of AR is important to clarify its etiology and develop appropriate preventive and therapeutic techniques. There have been few epidemiological studies on the age effect on the prevalence of AR and IgE sensitization to inhalant allergens, and serum total IgE levels in Japanese subjects. Therefore, we conducted an epidemiological study on a total of 1,540 subjects aged 20–49 years. The protocol comprised a questionnaire, measurement of total serum IgE antibodies and allergen-specific IgE antibodies against 7 aeroallergens in 2006 and 2007. The major findings of this study are the prevalence of allergic sensitization and AR, the age effect on them, and total serum IgE and AR, and the related age effect.

Material and Methods

Study Subjects

A total of 1,540 subjects were recruited from residents of Eiheiji-cho and the cities of Fukui, and Echizen in Fukui prefecture, in the central Hokuriku area of Japan in May and June of both 2006 and 2007. In that area, Japanese cedar pollen counts are at the average level of the islands of Honshu, Shikoku and Kyushu [7]. The 1,540 subjects were workers of 4 hospitals and students of nursing and medical colleges in the University of Fukui. The number of females was higher than that of males (mean age, 32.1 years; range, 20–49 years; male:female ratio, 1.0:2.40; mean serum IgE level, 233.8 IU/ml; median serum IgE level, 73.5 IU/ml). The participants were recruited during their annual health check-up in 2006 or 2007; 13 subjects did not agree to participate in this survey. Reasons for nonparticipation were lack of interest or time. All of the 1,540 participants agreed to measurement of serum total IgE and specific IgE to 7 aeroallergens and to answer a questionnaire. Blood collection and the questionnaire survey were performed at the same time after informed consent was received. We did not conduct a follow-up survey in this study. The diagnosis of AR was confirmed by seasonal or perennial symptoms of rhinitis consisting of any combination of the following: nasal itching, sneezing, discharge and stuffiness caused by inhalation of aeroallergens, reported on a questionnaire. All of the subjects with AR were also positive for serum-specific IgE to 1 or more of the 7 aeroallergens. All individuals were unrelated Japanese individuals and gave written informed consent to participate in the study according to the rules of the ethics committees of the Faculty of Medical Science, University of Fukui and the Institute of Physical and Chemical Research (RIKEN).

Measurement of Serum Levels of Specific IgE Antibodies

Specific IgEs to 7 aeroallergens, *Cryptomeria japonica*, *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f), *Dactylis glomerata*, *Ambrosia artemisiifolia*, *Candida albicans* and *Aspergillus fumigatus* were measured with a Pharmacia CAP System (Pharmacia CAP, Upsala, Sweden) (table 1). Allergen sensitization was classified as positive if the allergen-specific serum IgE level was above 0.7 (CAP RAST score of 2).

Statistical Analysis

To clarify the age-specific prevalence of AR and sensitization to the 7 aeroallergens examined, patients were divided into 3 age groups, the 20s (20 to <30 years), 30s (30 to <40 years) and 40s (40 to <50 years). We then compared differences in frequencies of sensitization to each of the 7 aeroallergens among these age groups by using the Kruskal-Wallis test and then by individual testing using the Mann-Whitney U test if significant. Serum total IgE was analyzed at a quantitative level, and log-transformed individual serum IgE levels were used in the figures. Correlations of total IgE levels and age were analyzed by Spearman's test. $p < 0.05$ was considered statistically significant. Logistic regression analysis was implemented for the AR and sensitization to assess the effects of gender, age and total serum IgE (SPSS 14.0J, SPSS, Inc., Chicago, Ill., USA).

Results

Prevalence of Allergic Sensitization and AR

Positive sensitization refers to an allergen-specific serum IgE level >0.7 (CAP RAST score of 2). The prevalence of allergic sensitization to each allergen tested is presented in table 1. Of the 1,540 subjects, 1,073 (69.7%) exhibited positive sensitization to at least 1 aeroallergen (fig. 1). A total of 467 of the 1,540 subjects (30.3%) showed

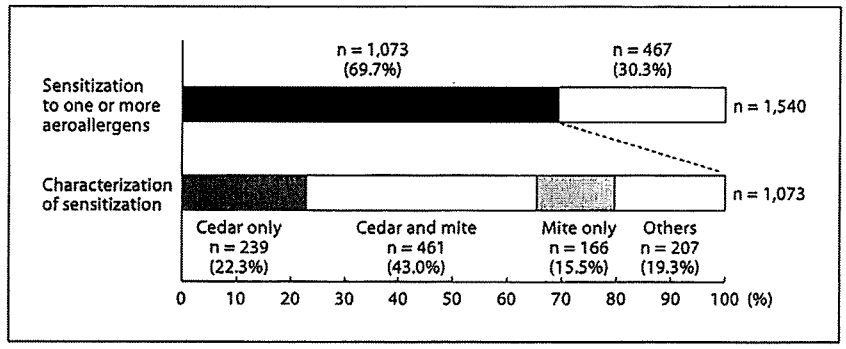

Fig. 1. The prevalence of sensitization to 7 test aeroallergens and characterization of sensitization.

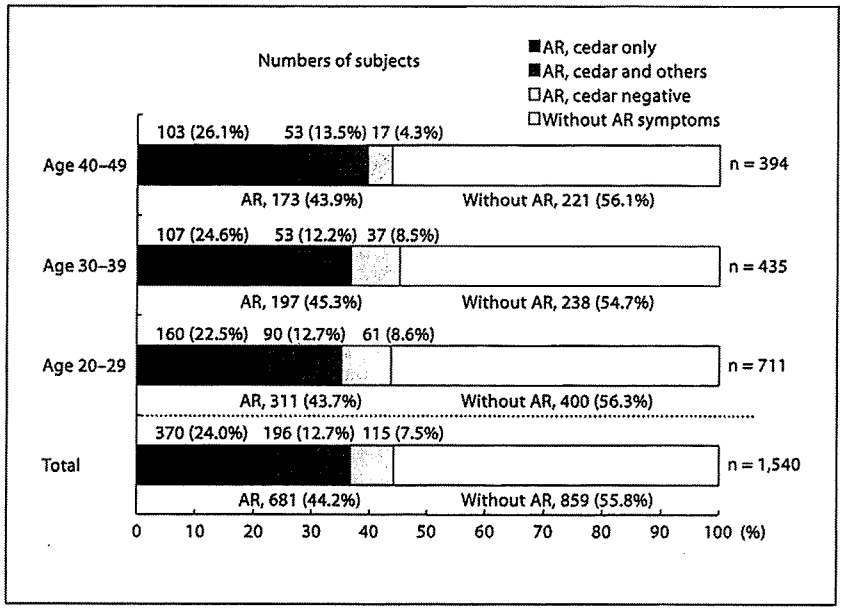

Fig. 2. Age effects on the prevalence of AR and sensitization to Japanese cedar pollen.

no sensitization to any of the 7 aeroallergens examined (fig. 1). Seven hundred subjects (45.3%) were sensitized to *C. japonica*, (Japanese cedar, JC) pollen, thus accounting for 65.3% of the 1,073 subjects with positive sensitization to aeroallergens. Of the 1,073 subjects, 627 (58.5%) were sensitized to mites. Thus, JC pollen and mites were the two predominant aeroallergens among the 7 tested aeroallergens (fig. 1).

Of the 1,540 participating subjects, 681 (44.2%) had symptoms of AR at the time of the survey (fig. 2). The prevalence of JCP was 36.7% (566 of the 1,540 subjects) in this study (fig. 2). The positive rates for specific IgE antibodies to Japanese cedar pollen were 89.6% (610 of 681) in the AR group and 28.5% (245 of 859) in the no-symptom group (fig. 3). Of the 681 AR subjects, 167 (24.5%) were sensitized to only Japanese cedar pollen (fig. 3).

Age Effect on the Prevalence of Allergic Sensitization and AR

We found significant associations between the allergic sensitization to the 7 aeroallergens and the age groups (table 1) ($p = 0.0019$ by the Kruskal-Wallis test). More subjects were sensitized to Japanese cedar pollen than to any other of the 7 tested allergens in each age group (table 1). The sensitization rates to Japanese cedar pollen were 59% (421 of 711 subjects), 52% (226 of 435) and 53% (208 of 394) for subjects in their 20s, 30s and 40s, respectively. We found a significant association between sensitization to Japanese cedar pollen and the age range of the subjects ($p = 0.015$ by the Mann-Whitney U test) (table 2). The sensitization rate against mites, Der p and/or Der f, was higher for those in their 20s (50%, 355 of 711 subjects), than for those in their 30s (41%, 179 of 435) and 40s

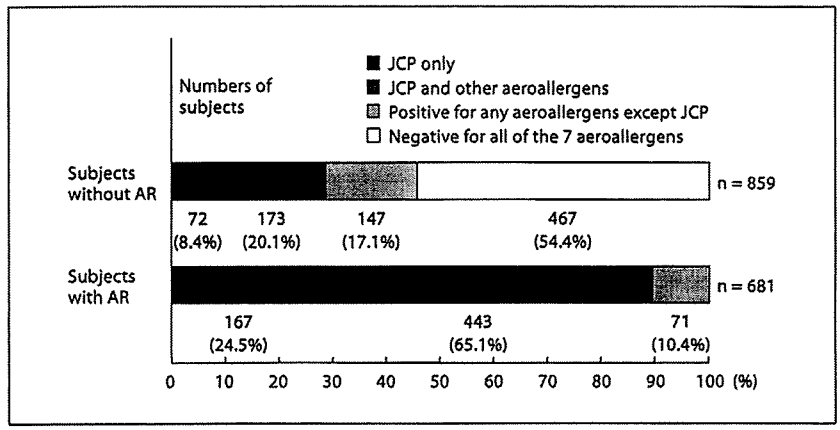

Fig. 3. Prevalence of AR and sensitization to Japanese cedar pollen.

Table 1. Prevalence of sensitization to 7 aeroallergens according to age group

	Total (n = 1,540)	20s (n = 711)	30s (n = 435)	40s (n = 394)
Cryptomeria japonica	855 (56)	421 (59)	226 (52)	208 (53)
Dermatophagoides pteronyssinus	625 (41)	345 (49)	174 (40)	106 (27)
Dermatophagoides farinae	622 (40)	342 (48)	168 (39)	112 (28)
Dactylis glomerata	352 (23)	198 (28)	90 (21)	64 (16)
Ambrosia artemisiifolia	137 (9)	67 (9)	45 (10)	25 (6)
Candida albicans	82 (5)	43 (6)	24 (6)	15 (4)
Aspergillus fumigatus	34 (2)	25 (4)	8 (2)	1 (0.3)

Figures in parentheses are percentages.

Table 2. Age effects on sensitization to JCP, dust mites and *Dactylis glomerata*

Aeroallergen	Sensitization	20s (n = 711)	30s (n = 435)	40s (n = 394)	p value
Cryptomeria japonica	positive	421 (59)	226 (52)	208 (53)	0.015
	negative	290 (41)	209 (48)	186 (47)	
Dust mites	positive	355 (50)	179 (41)	115 (29)	3.9×10^{-11}
	negative	356 (50)	256 (59)	279 (71)	
Dactylis glomerata	positive	198 (28)	90 (21)	64 (16)	4.8×10^{-6}
	negative	513 (72)	345 (79)	330 (84)	

Figures in parentheses are percentages. p value as obtained by the Mann-Whitney U test.

(29% 115 of 394), ($p < 0.001$ by the Mann-Whitney U test) (table 2). The prevalence of sensitization to *D. glomerata* was also higher in those in their 20s (28%, 198 of 711 subjects) than in those in their 30s (21%, 90 of 435) and 40s (16%, 64 of 394) ($p < 0.001$ by the Mann-Whitney U test) (table 2). AR was confirmed in 311 of the 711 subjects (43.7%) in their 20s, 197 of the 435 (45.3%) in their 30s and 173 of the 394 (43.9%) in their 40s (fig. 2). There was no significant difference in the prevalence of AR among the age groups.