欠く症例も存在する．その場合 PMR に GCA が合併していても GCA を診断するのは容易でない[10,11]．

今回，我々は PMR を発症した高齢女性に ^{18}F–fluorodeoxyglucose (FDG)–positron emission tomography (PET) を施行した際，大型血管炎の潜在を認めた症例を経験したので文献的考察を加えて報告する．

症　例

症　例：74 歳女性

主　訴：近位筋痛，発熱

家族歴：特記事項なし

既往歴：高血圧，脳梗塞

現病歴：2002 年より，間欠的な頭痛を自覚するようになった．2004 年 4 月 3 日突如全身の近位筋痛と 37℃ 台の発熱が出現．以後，症状が持続したため，2005 年 2 月 1 日当院を受診した．

初診時身体所見：体温 37.6℃．脈拍 76 回/分，不整なし．血圧 144/68 mmHg，左右差なし．眼底所見に異常を認めず，側頭動脈の拡張，圧痛も認められなかった．胸部聴診では呼吸音清，胸骨右縁第二肋間から右腋窩に放散する収縮期心血管雑音を聴取した．関節の腫脹は認められなかったが，頚部の強いこわばりと，上腕と大腿部に筋圧痛を認めた．また，右上下肢には，脳梗塞後遺症に伴う不全麻痺を認めた．

初診時検査所見（表 1）：赤沈 90 mm/hr と亢進，CRP 6.6 mg/dl と陽性．また，CPK の上昇は認めず，リウマチ因子陰性，抗核抗体陰性であった．血液培養陰性．胸部単純 X 線写真では肺野に異常所見は認められなかった．また，大動脈弓の石灰化は無く，さらに大動脈の蛇行も認められなかった（図 1）．心エコーでは軽度の僧帽弁閉鎖不全症を認めたが，大動脈弁，肺動脈弁に異常は認められなかった．また，明らかな vegetation も認められなかった．

臨床経過：高齢，突然発症の発熱，近位筋痛，さらに赤沈の亢進，CRP の上昇から本症例を PMR と診断した[1]．新たに発症した頭痛のエピソードがあったが，側頭動脈に圧痛や拡張を認めず，眼底所

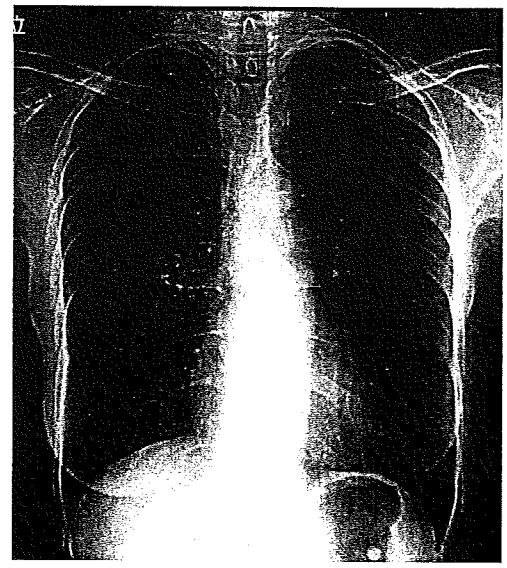

図 1　胸部単純 X 線写真

表 1　初診時検査所見

赤沈	一時間値	90 mm	生化学	TP	6.8 g/dl	免疫検査	CRP	6.6 mg/dl
				Alb	4.0 g/dl		STS	(−)
検尿	潜血	(−)		T. Bil	0.9 mg/dl		TPHA	(−)
	蛋白	(−)		GOT	13 IU/l		HBsAg	(−)
	糖	(−)		GPT	19 IU/l		HCV 抗体	(−)
	沈渣	異常なし		LDH	182 IU/l		IgG	1259 mg/dl
				ALP	240 IU/l		IgA	336 mg/dl
血算	WBC	5700/μl		CPK	79 IU/l		IgM	80 mg/dl
	Neutro	73%		AMY	45 IU/l		RF	陰性
	Eosino	1%		Cr	0.5 mg/dl		抗核抗体	陰性
	Baso	1%		BUN	11 mg/dl		抗 SS-A 抗体	陰性
	Mono	7%		Na	141 mEq/l		PR3-ANCA	陰性
	Lymph	18%		K	4.0 mEq/l		MPO-ANCA	陰性
	RBC	400×10^4/μl		Cl	107 mEq/l		CH_{50}	60 mg/dl
	Hb	11.4 g/dl		Glucose	104 mg/dl			
	Ht	34.2%				HLA typing	A24, 26. B60, 61	
	Plt	27.4×10^4/μl	細菌培養	血液培養	陰性		DR4, 9	

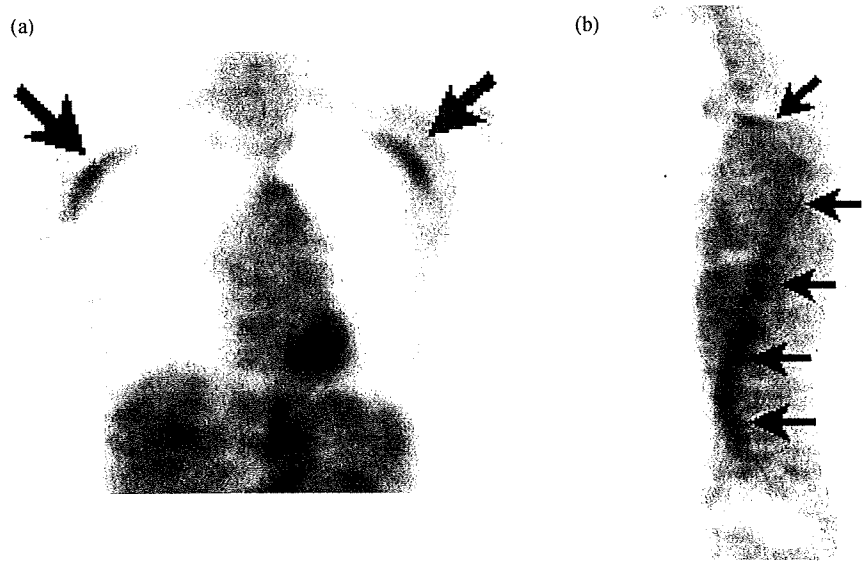

図2　^{18}F–FDG–PET像
a）正面像（左）
b）側面像（右）
大動脈および両側鎖骨下動脈に強いFDGの集積（矢印）を認めた．

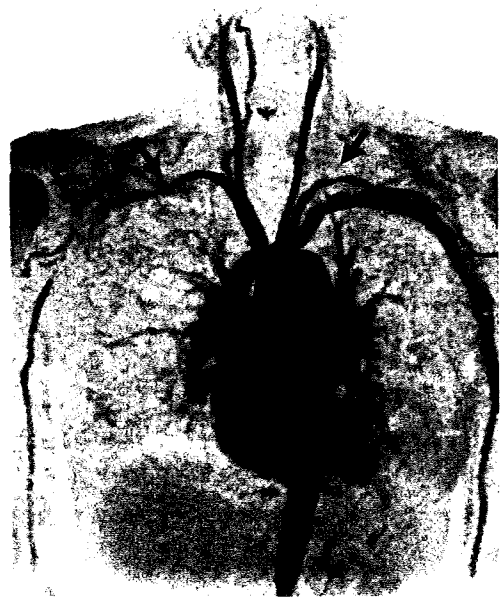

図3　胸部造影MRA像
両側鎖骨下動脈の狭窄（矢印）を認めた．

見も正常な事からGCAの合併は当初否定的と考えた．他疾患の除外，特に悪性腫瘍の潜在の有無評価のため，2005年2月22日^{18}F–FDG–PETを施行した．その結果，悪性腫瘍を疑わせる所見は無いものの，大動脈および両側鎖骨下動脈にFDGの集積を認め大型血管の炎症が強く疑われた（図2-a,b）．2005年3月2日磁気共鳴血管造影（Magnetic resonance angiography；MRA）を施行したところ，両側鎖骨下動脈の狭窄を認め大型血管炎の所見に矛盾しなかった（図3）．また，心血管雑音の原因は，これらの動脈狭窄によるものと考えられた．PMRと合併した大型血管炎に対して，2005年3月11日よりPrednisolone（PSL）20 mg/日の投与を開始した．ステロイド開始後，速やかに解熱．筋痛も改善し，CRPは陰性化，聴診上収縮期心血管雑音も減弱した．その後，1年の経過でPSLを10 mgまで漸減．2007年4月PSLを8 mgに減量したところ，発熱，筋痛の再燃を認め，2008年8月現在PSL10 mg維持投与中である．

考　察

側頭動脈の圧痛や視力低下などの頭蓋症状を欠くPMRの症例にFDG–PETを施行したところ，大型血管炎の潜在を認めた．PETは，生体の代謝レベルを観察することに特化した検査法である．FDG–PETは，腫瘍組織における糖代謝レベルの上昇を検出することにより，悪性腫瘍の診断に広く臨床応用されている．また，FDGは腫瘍組織ばかりでなく，ブドウ糖代謝の盛んな正常組織や炎症部位にも集積する[12]．PETでの動脈壁へのFDGの集積は，大型血管炎で多く報告されている[10,13,14]．血管炎以外の病態としては，動脈硬化でも血管壁へのFDGの集積が認められる事がある．しかし，動脈硬化に伴うFDG集積例の多くは大動脈，腸骨動脈，さらに大腿動脈への集積で，動脈硬化の好発部位に一致する[15]．本症例では動脈硬化は軽度で，大動脈と上

表2 大型血管炎の鑑別

	高安動脈炎	巨細胞性動脈炎	本症例
障害される血管	大動脈と第一分枝	大動脈と第一分枝 外頸動脈の分枝	大動脈と鎖骨下動脈
好発年齢	40歳以下	50歳以上	74歳
頭痛	まれ	高頻度	あり
PMR*の合併	まれ	高頻度	あり
HLAの特徴	B52陽性	DR4陽性	B60, 61 DR4, 9

* PMR：リウマチ性多発筋痛症

肢に分岐する動脈を中心にFDGの集積と狭窄病変を認める事から，FDGの集積に動脈硬化が関与した可能性は低いと考えられた．

一方，本症例の大型血管炎の鑑別には，GCAと高安動脈炎（Takayasu arteritis）が挙げられる．Chapel Hillの血管炎分類[16]では，大型血管炎（large-vessel vasculitis）は大動脈やその第一分枝が標的血管となる血管炎と定義され，これらの疾患で障害される血管は共通である．しかし，GCAと高安動脈炎では発症年齢や臨床症状，さらにHLA等に相違がみられる（表2）．

GCAは50歳以上に発症し[17]，典型例では側頭動脈に発赤腫脹や圧痛さらに拍動の減少などの異常所見を認める．眼動脈が侵されるケースでは虚血性の視神経炎を呈し失明をきたす事がある．また，限局性頭痛は2/3の症例に認められ[5,6]，赤沈値は亢進し，PMRを高頻度（40-60%）[2~4]に合併する．さらに側頭動脈生検では巨細胞を伴った動脈炎を認める．HLAはDR4陽性例が多い[18]．一方，高安動脈炎は40歳以下に発症し，発熱，視力低下，四肢の跛行，上腕動脈拍動低下等の臨床症状を呈する[19]．HLAはB52陽性例が多い[20]．本症例は高齢発症で，新たに発症した頭痛を伴い，PMRを合併，さらにHLA B52陰性，DR4陽性でGCAに特徴的な所見と一致した．確定診断には側頭動脈生検が必要だが，頭蓋症状を欠く症例での側頭動脈生検陽性率は58%に留まる[11]．本症例では患者の同意が得られず側頭動脈生検は施行できなかった．大型動脈の血管炎の診断には血管造影が有用であり，本症例ではFDG-PETでの大血管へのFDG集積をきっかけにMRAを施行し鎖骨下動脈の狭窄病変が証明された．以上より本症例に合併した大型血管炎はGCAであると考えられた．

PMRは高齢者に発症するリウマチ性疾患であるが，GCA合併の有無により治療反応性と予後が異なる．PMRは少量のステロイド（PSL10~20 mg）で臨床症状は速やかに改善する[21]．ステロイドの中止により50%の再発症例があるものの，緩徐な減量によりステロイドの投与が中止できる症例が多い[22,23]．また，再発例でも少量のステロイド再投与で寛解する例が多い．一方，GCAではステロイド大量療法（PSL40~60 mg）により臨床症状は改善するが，経過中60%の症例で再発を認める[24]．本症例でもPSL10 mgの維持投与をしているが，さらなるステロイドの漸減が困難である．再発例ではステロイドの増量や免疫抑制剤の併用が必要となる場合が多い．さらに，GCAに生じる大型血管病変では血管壁の弾性膜が破壊され，胸部大動脈瘤が発生する可能性が健常人と比較すると17倍高く，大動脈解離や動脈瘤の破裂に注意を要す[25]．以上のようにステロイド治療後の漸減過程でPMRはGCA合併の有無により異なる経過をとる．よって，たとえ診断時に明らかなGCAの合併が証明できなくても，治療抵抗例やステロイド漸減困難例なPMRでは，大型血管炎の潜在を念頭におき血管造影やFDG-PETによる積極的な血管炎の検索を検討すべきと考えられる．

また，GCAを併発したPMRのステロイド治療量は，本来ならばGCAに対するステロイド量を充てるのが妥当と考えられる．しかし，本症例は臓器虚血症状に乏しく患者が外来加療を強く希望されたため，外来で比較的安全に投与が可能なPSL20 mgを初期治療量として用いて幸い寛解した．

PMRでは側頭動脈の圧痛や拡張等の臨床症状を伴わなければGCAの診断は困難で，特に本症例のように大型血管に潜在した血管炎は見逃される可能性が高い．しかし，大型血管に潜在するGCAでは上肢の跛行や，本症例のように血管雑音（80%）を認め[11]，診断には頭蓋症状だけでなく上肢の血行障害の有無を問診や診察で確認する事が重要である．

また，Blockmansらは側頭動脈の生検が陰性のPMR35例のうち，30%の症例にFDG-PETで主に鎖骨下動脈にFDGの集積を認めたと報告している[14]．この結果は本症例のように頭蓋症状に乏しいPMRにおいて，大型血管に限局したGCAの潜在は稀ではない事を示唆している．一方で血管壁へのFDGの集積は，動脈硬化を生じる高齢者にも多く認められ，集積部位や血管造影による血管の狭窄や動脈瘤の有無を加味した上で鑑別する必要があると考えられた．

結　語

FDG-PETがきっかけで，大型血管炎の合併を診断し得たPMRの1例を経験した．FDG-PETは悪性腫瘍の検索だけでなく，PMRに潜在する大型血管炎の評価に有用であると考えられた．

本論文の要旨は第16回日本リウマチ学会関東支部学術集会（2005年12月，筑波）において発表した．

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Online publication June 24, 2009

日本人集団における顕微鏡的多発血管炎の疾患感受性遺伝子解析

土屋　尚之

要　旨：筆者らは，日本人顕微鏡的多発血管炎(MPA)の疾患感受性と関連する遺伝子多型を探索した。*HLA*の解析からは，日本人では頻度が高く，ヨーロッパ系集団には存在しない*HLA-DRB1*0901-DQB1*0303*ハプロタイプの有意な増加が見いだされた。また，*KIR*およびそのリガンドである*HLA*の組み合わせの解析により，抑制型シグナルが優位と想定される*HLA-KIR*の組み合わせが増加していた。さらに，*LILRA2*のスプライス部位多型との関連が見いだされた。今後，大規模な研究による確認が期待される。(J Jpn Coll Angiol, 2009, **49**: 31–37)

Key words: microscopic polyangiitis, genetics, HLA, KIR, LILRA2

はじめに

ANCA関連血管炎のなかで，欧米，特に北部ヨーロッパ集団ではWegener肉芽腫症(Wegener's granulomatosis: WG)が多いのに対し，日本では，顕微鏡的多発血管炎(microscopic polyangiitis: MPA)が多い。このような集団間の違いには，何らかの遺伝因子，環境因子が関与すると推測される。可能性のある環境因子の一つとしては微生物感染が想定され，ヒトのANCA関連血管炎では，*Staphylococcus aureus*[1]，サイトメガロウイルス(CMV)感染[2]などの関与を示唆する報告がある。また，本特集の他項には，血管炎モデル動物において，微生物の寄与を示唆する研究が紹介されている。現状では，ANCA関連血管炎は，微生物などの環境因子に対する応答の個体差が発症に関与する，との考え方が適切と思われる。

ANCA関連血管炎のように病因解明の手がかりに乏しい疾患では，疾患感受性遺伝子探索が病因や本質的病態の解明，治療法の開発に手がかりを与えることと期待される。しかし，疾患の稀少性のために，ゲノムワイド連鎖解析のために必要な疾患多発家系や，信頼性の高いゲノムワイド関連解析に必要な，数百人以上という患者検体を収集することは事実上不可能であり，ヒトにおける病態解析や動物モデルに基づく成果を利用した候補遺伝子アプローチに頼ることになる。

免疫系遺伝子群は，生体の環境応答の主要な因子であり，微生物という選択圧により，人類集団において，顕著な機能的多様性が獲得されていることから，有力な候補遺伝子と考えられる。筆者らは，厚生労働省研究班「難治性血管炎に関する調査研究班」の研究分担者として，日本人ANCA関連血管炎の遺伝的背景について，免疫系遺伝子を候補遺伝子として検討を加えた。多施設共同研究であったものの，疾患の稀少性のために，解析し得た検体は，MPA 50，WG 8，Churg-Strauss 症候群 8という少数であったため，主としてMPAを解析対象とした。ANCA関連血管炎の遺伝素因に関しては，国内外を含めて研究は少数であり，さらに，そのほとんどを占める欧米と日本では，上述のように，ANCA関連血管炎の疫学はかなり異なることから，本稿では，ANCA関連血管炎全般の総説ではなく，研究班における筆者らの成果を紹介することとする。

*HLA*領域遺伝子

HLAは，T細胞に対する抗原提示分子であり，直接免疫応答に影響する顕著な多型が認められる。多くの免疫

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2008年 8 月20日受理

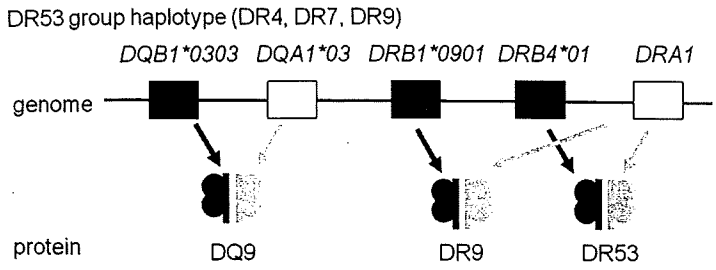

Figure 1 Possible mechanisms of association between *HLA-DRB1*0901-DQB1**0303 haplotype with MPA. *HLA-DRB1*0901-DQB1*0303* is one of the haplotypes that belong to DR53 group haplotype, which carries *HLA-DRB4* locus. Three HLA-class II molecules, HLA-DR9, DQ9 and DR53, are produced from this haplotype. It is possible that an antigenic peptide specifically presented by any of these molecules is causally involved in diseases. Another possibility is as yet unidentified functional polymorphism specifically encoded on this haplotype is causally associated with diseases, and HLA represents a proxy of such causal allele.

Possible mechanisms of association with MPA
(1) Presentation of specific antigenic peptides by HLA molecules encoded by this haplotype such as DQ9, DR9 and DR53.
(2) Functional polymorphisms of other genes encoded on this haplotype

疾患や感染症において，*HLA*はもっとも確立した疾患感受性遺伝子であるため，本研究においても，まず，*HLA*を候補遺伝子として解析した。

HLAとANCA関連血管炎の関連研究では，ヨーロッパにおいて，WGとHLA-DR4, DR1との関連を示唆する報告がみられる[3~5]。日本では，筆者らの研究以前に，少数例の検討であるが，WGおよびMPO-ANCA関連腎炎におけるHLA-DR9の増加が報告されていた[6,7]。

筆者らは，日本人ANCA関連血管炎と*HLA-DRB1*遺伝子型との関連解析を施行し，*HLA-DRB1*0901*が，健常対照群の29.1%に対し，MPAの50%に陽性であり，有意に増加していることを見出した($P = 0.0037$, オッズ比[OR]2.44)[8]。さらに，*HLA-DQB1, DPB1, B, C*についての検討も加えたところ，*DQB1*0303*に対してもOR 2.35の有意な関連が検出された。*DRB1*0901*と*DQB1*0303*の間には顕著な連鎖不平衡が存在するため，これらのいずれが一義的な感受性遺伝子かを決定することは不可能であった。一方，*HLA-B, C, DPB1*には，明らかな関連は検出されなかった。すなわち，日本人集団において，*HLA-DRB1*0901-DQB1*0303*ハプロタイプは，MPAの有意な遺伝因子であることが明らかになった[9]。

*HLA-DRB1*0901-DQB1*0303*ハプロタイプには，ユニークな特徴が2点存在する。第一は，このハプロタイプは，日本をはじめとするアジア集団にはきわめて高頻度に存在するのに対し，ヨーロッパ系集団，アフリカ系集団には，ほとんど存在しないという点であり，第二は，このハプロタイプは，日本人において，I型糖尿病，若年型重症筋無力症，抗リン脂質抗体産生，抗CCP抗体陰性関節リウマチなど，多彩な自己免疫疾患とも関連が認められるという点である[10~13]。あたかも，北部ヨーロッパ集団に高頻度に存在するが，日本人には存在せず，各種自己免疫疾患との関連が認められる*HLA-DRB1*0301*ハプロタイプと鏡像の関係ということができる。

HLA-DR, DQの機能は，T細胞に対する抗原提示である。従って，*HLA-DRB1*0901-DQB1*0303*ハプロタイプとMPAとの関連を説明する分子機構の一つの可能性は，*HLA-DRB1*0901*遺伝子と*HLA-DRA1*遺伝子のヘテロダイマーであるHLA-DR9分子，*HLA-DQB1*0303*と，このハプロタイプにコードされた*DQA1*03*の遺伝子産物のヘテロダイマーであるHLA-DQ9分子，さらにこのハプロタイプにコードされている*HLA-DRB4*01*と*DRA1*の遺伝子産物のヘテロダイマーであるHLA-DR53分子のいずれかに，何らかの病因的抗原ペプチドが特異的に提示されるというものである(**Fig. 1**)。

しかし，上記のように多彩な疾患との関連を，特異的抗原ペプチドの結合で説明するのは，やや困難であるように思われる。*HLA*遺伝子群は，ヒト6p21.3にコードされているが，この領域には，TNFα, C4, TAPなど，HLA以外の免疫系遺伝子が多数コードされており，HLAとの連鎖不平衡が認められる。このハプロタイプがさまざまな自己免疫疾患に関連することを考慮すると，特異的な抗原ペプチドの提示よりも，むしろ，このハプロタイプに特異的にコードされた，HLA以外の免疫系遺伝子の多型が原因的である可能性が高いように思われる(**Fig. 1**)。今後，これら両方の可能性を考慮に入れて，分子機構を検証していく必要がある。

また，前述のように，WGとMPAの有病率には，顕著

な集団差が認められるが，この原因がHLAの集団差であるか否かについても，今後，さまざまな集団の検討により，検証していく必要がある。

*KIR*遺伝子群

ヒトNK細胞は，多数の活性化型受容体と抑制型受容体を発現している。抑制型受容体は，大部分の細胞に発現する自己のHLA-class I分子を認識し，抑制性シグナルを伝達することにより，NK細胞による自己の細胞傷害性を防いでいるが，腫瘍細胞やウイルス感染細胞など，自己のHLA-class I発現が抑制されるような状況においては，抑制型シグナルが減弱し，そのような細胞に対する細胞傷害性が発揮される(missing self説)[14]。免疫グロブリンスーパーファミリーに属するkiller cell immunoglobulin-like receptor(KIR)は，NK細胞や一部のT細胞に発現する主要な受容体であり，活性化型，抑制型両方の受容体が存在する。

KIRは，ヒト染色体19q13.4に位置するleukocyte receptor complex(LRC)に，leukocyte Ig-like receptor (*LILR*)遺伝子群に隣接して存在する14の機能遺伝子座にコードされている。KIRは，細胞外のIgドメインの数と細胞内領域の長さによって命名される。例えば，KIR3DL1は細胞外に3個のIgドメインを有し，長い細胞内領域を持つ分子であり，KIR2DS1は細胞外Igドメインが2個で，細胞内領域が短い分子である。一般に，DLのグループは，細胞内領域に抑制型モチーフであるimmunoreceptor tyrosine-based inhibitory motif(ITIM)を有する抑制型受容体であり，DSのグループは，膜貫通領域に陽性荷電のアミノ酸(Lys)を有し，活性化型モチーフであるimmunoreceptor tyrosine-based activation motif (ITAM)を有するアダプター分子であるDAP12と会合し，活性化型シグナルを伝達する。ただし，DLのグループの中でKIR2DL4のみは，膜貫通領域に陽性荷電のArgを有し，FcRγと会合してIFNγ産生を誘導することが報告されている[15]。

KIRは，T細胞受容体と異なり，特定の抗原ペプチドを提示したHLAのみと反応するわけではなく，アミノ酸配列モチーフによって分類されるHLA-class Iのサブグループを認識する(**Table 1**)。

*KIR*遺伝子群には，塩基配列レベルでの多型に加え，遺伝子座自体の有無による多型が顕著に存在する。この結果，ある個人がゲノム中に有する*KIR*遺伝子の数には，7～14種類という大きな個体差が存在する。このような多様性の詳細については，総説を参照されたい[16~18]。

Table 1 KIRs and their ligands

KIR	ligand
KIR2DL1	HLA-Cgroup2
KIR2DL2/3	HLA-Cgroup1
KIR2DL4	HLA-G
KIR2DL5	
KIR3DL1	HLA-Bw4
KIR3DL2	HLA-A3, A11
KIR3DL3	
KIR2DS1	HLA-Cgroup2
KIR2DS2	(HLA-Cgroup1)
KIR2DS3	
KIR2DS4	
KIR2DS5	
KIR3DS1	(HLA-Bw4)

HLA-Cgroup2 consists of HLA-Cw alleles possessing Asn77 and Lys80, such as Cw2, 4, 5 and 6. HLA-Cgroup1 includes HLA-Cw1, 3, 7 and 8 which contain Ser77 and Asn80. HLA-Bw4 is defined by position 77-83 amino acids, and includes HLA-B13, B27, B44, B51, and B52. Weak interaction has been postulated between KIR2DS2 and HLA-Cgroup1, and between KIR3DS1 and HLA-Bw4, which has not been conclusively established.

*KIR*と*HLA*は，別の染色体にコードされているために，独立に遺伝する。従って，いずれも高度に多型的である*KIR*とリガンドである*HLA*の組み合わせにより，HLA-KIRを介するシグナル伝達に遺伝的個体差が生じる。以上のような背景から，KIRとHLAの組み合わせと，特にHIV[19]，HCV[20]，ヒトパピローマウイルス(HPV)[21]などのウイルス感染，関節リウマチ[22]，尋常性乾癬[23]，乾癬性関節炎[24]，I型糖尿病[25]，強皮症[26]などの自己免疫疾患との関連が報告されている。これまでの研究成果を大きくまとめると，HLA-KIRの組み合わせから想定される活性化型シグナルが強い場合に自己免疫疾患の，抑制シグナルが強い場合にウイルス感染抵抗性減弱のリスクの上昇の傾向が認められる例が多いが，ウイルス感染が発症に関与する子宮頸癌では抑制型の組み合わせの方が疾患抑制的に働き，これはウイルス感染に対する宿主の炎症反応の持続が発癌に関連するためであると解釈されている。

一個体の中の個々のNK細胞は，その個体の持つ*KIR*

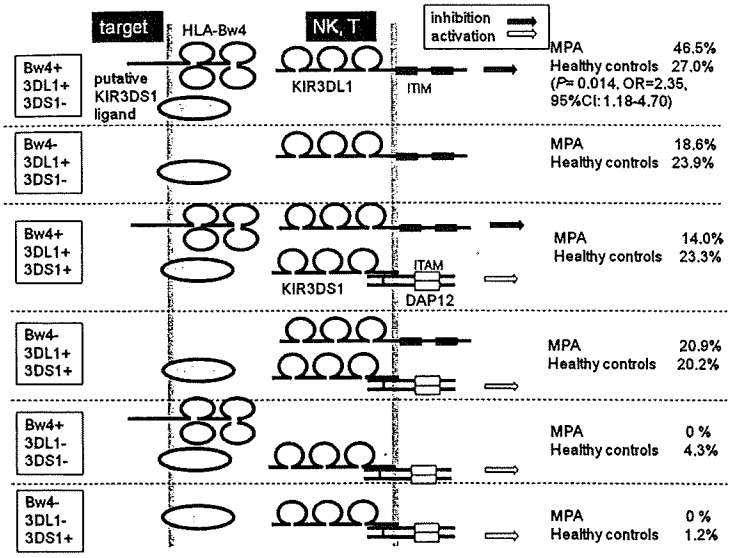

Figure 2 *HLA-Bw4, KIR3DL1 and KIR3DS1* combinations in Japanese MPA and healthy controls.
Each individual can be grouped into 6 groups according to the presence/absence of *KIR3DL1*, *KIR3DS1* and *HLA-Bw4*, the ligand of KIR3DL1 (and possibly of KIR3DS1). KIR3DL1 contains ITIM and transmits inhibitory signal, while KIR3DS1 associates with DAP12 which contains ITAM, and transmits activation signal. Frequencies of each group in the Japanese patients with MPA and controls are shown on the right. The most inhibitory combination, HLA–Bw4+, KIR3DL1+, KIR3DS1− (top), was significantly increased in MPA.[28] Because the ligand of KIR3DS1 has not been identified, here we assumed an unknown ligand expressed in all individuals. It should be noted that there is a possibility that KIR3DS1 weakly interacts with HLA-Bw4, as described in Table 1. However, this does not significantly affect the above interpretation.
OR: odds ratio, CI: confidence interval.

ファミリー遺伝子群を，ある程度ランダムに発現する。従って，同じ個体のNK細胞の中においても，あるKIRを発現する細胞と発現しない細胞が存在する。CMV感染の反復する1例の小児において，すべてのNK細胞が抑制型受容体であるKIR2DL1を発現していたという現象が報告されている[27]。これは，KIRを介する抑制シグナルが強いと想定される個体において，ウイルス感染抵抗性が減弱することを支持する知見である。

以上の背景から，われわれは，日本人MPAにおける*KIR*遺伝子の有無とHLAリガンドとの組み合わせの解析を行った[28]。

まず，日本人MPA43例，健常対照者239例において，*KIR*の14遺伝子座の有無を検討したところ，活性化型受容体である*KIR2DS3*の陽性率が健常群の16.7%に比べMPAでは4.7%と有意に減少していた($P=0.038$)。

次に，HLAリガンドとKIRとの組み合わせを検討した。HLA-B，HLA-C単独ではMPAと健常群に統計学的に有意な差は見られなかった。しかし，*KIR3DL1/3DS1*と*HLA-Bw4*との組み合わせのうち，もっとも抑制的であると予想される，*HLA-Bw4*陽性，*KIR3DL1*陽性，*KIR3DS1*陰性という群が，MPAにおいて46.5%と，健常群の27.0%と比較してオッズ比2.35で有意に増加していた($P=0.014$)(**Fig. 2**)。

この組み合わせに加え，さらにHLA-Cをリガンドとする組み合わせをあわせて考慮したところ，この解析においても，より抑制的と思われる組み合わせほどMPAに対するリスクが上昇する傾向が観察された。以上の結果から，KIRを介するNK細胞・T細胞の抑制がMPA発症リスクに関連する可能性が示唆された。

この結果を前述の自己免疫疾患やウイルス感染との関連に関するこれまでの報告と考え合わせると，自己免疫疾患よりもむしろ，ウイルス抵抗性の減弱と関連する組み合わせがMPAに感受性であるという可能性が示唆される。冒頭に述べたように，MPAをはじめとするANCA関連血管炎において，*Staphylococcus aureus*やCMVが原因的に関与する可能性を示唆する報告が存在する[1,2]。また，MPAが高齢発症の疾患であることや，経過中にCMV肺炎をはじめとする重症難治性感染症の合併が多いことなどを考え合わせると，*KIR*遺伝子型による感染抵抗性の遺伝的な減弱が存在する個体に，加齢によるさらなる抵抗性の減弱が加わり，ウイルス感染の遷延化・重症化がもたらされ，これが複数の遺伝素因や環境要因の影響を受けてANCA産生につながり，血管炎の発症に至るとの仮説を提唱し得るのではないかと思われる。

LILRA2

*KIR*遺伝子群と隣接してLRCに位置する*LILR*(*ILT*, *CD85*とも呼ばれる)遺伝子群にも，2個の偽遺伝子を含めて13遺伝子座が存在する。LILRは，活性化型(LILRA1, -A2, -A4, -A5, -A6)，抑制型(LILRB1, -B2, -B3, -B4, -B5)および可溶型(LILRA3, -A5s)の3群に分類される。個々のLILRによって分布は異なるものの，一般的には，白血球系に広汎に発現し，一部のLILRは，HLA-class IやCMVのUL18をリガンドとするこ

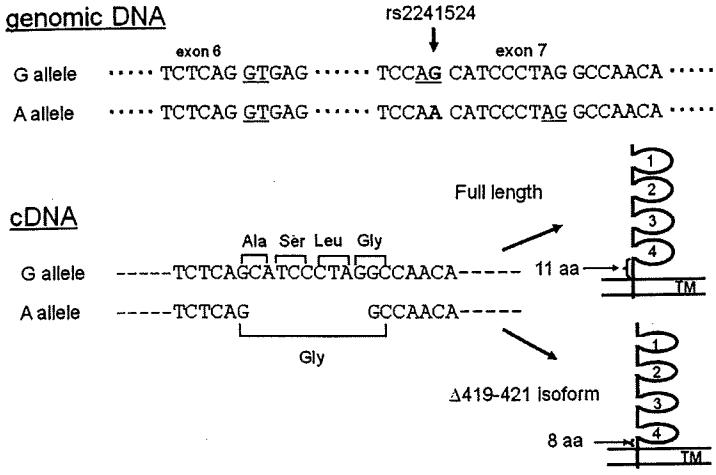

Figure 3 *LILRA2* rs2241524 associated with MPA and SLE activates a cryptic splice acceptor site and causes in-frame deletion of three amino acids in the linker region. The SNP rs2241524 G > A (arrow) disrupts the consensus splice acceptor motif, and activates cryptic splice site 9 nucleotides downstream of the original splice acceptor site.[29] This leads to inframe deletion of 3 amino acids (419–422) in the linker region of LILRA2. The splice sites employed in each allele are underlined, and exons are shaded.

とが知られている。

これらのうちで，LILRA2は，好酸球，好塩基球やマクロファージの活性化に関与することが推測されている。一方で，単球におけるIL-12産生をIL-10にシフトさせ，TLRシグナルを抑制することにより，免疫応答に抑制的に働くとの報告もある。

筆者らは，*LILR*遺伝子群を候補遺伝子と考え，リウマチ膠原病における関連研究を進める過程で，*LILRA2*のintron 6-exon 7 junctionのスプライス受容部位を置換するSNPと全身性エリテマトーデス(SLE)，MPAとの関連を見いだした[29]。すなわち，A/A遺伝子型が，健常対照群の7.0%に対し，SLEでは12.1%(P = 0.041，G/G遺伝子型を対象とした場合のOR 1.82)，MPAでは 16.0%(P = 0.049，OR 2.52)と，有意に増加していた。

このSNPはスプライシング部位を変更することが予測されたため，それぞれの遺伝子型において，cDNA配列を検討したところ，Aアリルを有する場合，9 塩基下流に存在するcryptic splice siteが利用されるため，アミノ酸配列上，position 419–421に相当する 3 アミノ酸が欠失したタンパク質に翻訳されることが想定された(**Fig. 3**)。A/A遺伝子型の個体では，100%のmRNAがこのΔ419–421 isoformであった。Position 419–421は，LILRA2分子において，細胞外ドメインのIg-likeドメインと膜貫通領域の間に存在するlinker部位に相当する位置に存在する。モノクローナル抗体を用いた検討により，このアイソフォームも単球表面上に表出していることが確認された。この 3 アミノ酸の欠失により，LILRA2シグナルがどのように変化するかに関しては，今後の検討が必要である。

結　語

われわれは，MPAの遺伝素因に関して，*HLA*，*KIR*，*LILR*という，免疫系の遺伝子群の中でも特に機能的多型に富む多重遺伝子ファミリーを中心に，検討を行ってきた。稀少疾患であり，遺伝学の研究としてはきわめて少数例の検討であることから，その再現性の確認は，今後の検討を待たねばならないが，いずれの遺伝子においても，他の自己免疫疾患や感染症との関連が，過去の報告あるいは筆者ら自身の研究により見いだされていることは，偽陽性であるよりも，真の関連である可能性を支持する知見と思われる。

今後，本研究から見いだされた関連の確認とともに，分子機構の検討を進め，病因解明や創薬につなげることが期待される。さらに，数百というレベルで日本人ANCA関連血管炎の検体収集を行うことが可能であれば，ゲノムワイド関連研究により，網羅的な疾患感受性遺伝子解析を行うことも可能になる。今後の全国レベル，さらには，アジア集団における共同研究体制の構築が期待される。

謝　辞

本研究は，「難治性血管炎に関する調査研究班」の研究分担者として，厚生労働科学研究費補助金の援助のもとに施行されました。研究代表者を務められ，本研究の機会を与えて下さいました橋本博史先生(順天堂大学)，尾崎承一先生(聖マリアンナ医科大学)，ならびに本研究に多大な御指導，御協力を下さいました多数の先生方に，この場をお借りして深謝申し上げます。

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Genetics of Microscopic Polyangiitis in the Japanese

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Key words: microscopic polyangiitis, genetics, HLA, KIR, LILRA2

The epidemiology of ANCA-associated vasculitis is substantially different between Caucasians and Japanese, which may be related to genetic background. In this review, I discussed our findings on the genetics of microscopic polyangiitis (MPA) in Japanese. Analysis of *HLA* genes revealed significant increase of *HLA-DRB1*0901-DQB1*0303* haplotype in MPA. This is one of the most frequent haplotypes in Japanese, but virtually absent in Caucasians, and has been shown to be associated with multiple autoimmune diseases.

Analysis of *KIR* genes revealed significant decrease in the carrier frequency of an activating receptor *KIR2DS3* in MPA. When *KIRs* were analyzed in combination with HLA ligands, the proportion of individuals carrying *KIR3DL1* and *HLA-Bw4* but not *KIR3DS1*, the most inhibitory of all *KIR3DS1/3DL1/HLA-B* combinations, was significantly increased in MPA. These results suggested that the decreased activation of NK and/or T cells may predispose to MPA.

LILRA2 is an activating receptor involved in granulocyte and macrophage activation. *LILRA2* SNP rs2241524 G > A that disrupts intron 6 splice acceptor site was significantly associated with MPA. The risk allele produces a LILRA2 isoform lacking three amino acids in the linker region.

These findings, when confirmed by larger-scale studies, will shed light on the molecular mechanisms of MPA.

(J Jpn Coll Angiol, 2009, **49**: 31–37)

February 25, 2009

Online publication June 24, 2009

37

Replication of association between *FAM167A(C8orf13)-BLK* region and rheumatoid arthritis in a Japanese population

Polymorphisms in the genomic region encoding B lymphoid tyrosine kinase (*BLK*) and family with sequence similarity 167, member A (*FAM167A*, also referred to as *C8orf13*) at 8p23.1 have been associated with systemic lupus erythematosus (SLE) in Caucasian[1 2] and Asian[3 4] populations. A recent genome-wide study in a north American population showed new associations with rheumatoid arthritis (RA), among which was a single nucleotide polymorphism (SNP) rs2736340 in the intergenic region of *BLK* and *FAM167A*.[5] In the HapMap Japanese samples (http://www.hapmap.org/index.html.ja), this SNP is in absolute linkage disequilibrium (r^2=1) with rs13277113, previously associated with SLE.[1–4] We have shown that the population frequency of the risk genotype rs13277113A/A and the OR for SLE were substantially higher in the Japanese population than in the Caucasian population.[3]

Table 1 Association of *BLK* rs13277113 with rheumatoid arthritis (RA) in a Japanese population

		Genotype frequency			Allele frequency		Allelic association	
	n	A/A	A/G	G/G	A	G	p Value	OR (95% CI)
RA	603	308 (0.511)	242 (0.401)	53 (0.088)	858 (0.711)	348 (0.289)	0.018	1.24 (1.04 to 1.49)
Control	492	218 (0.443)	218 (0.443)	56 (0.114)	654 (0.665)	330 (0.335)		

The association was tested by χ^2 analysis using a 2×2 contingency table.

To date, the association of *FAM167A-BLK* region with RA has not been reported in non-Caucasian populations. In this study we examined whether the association between *BLK* and RA was replicated in Japanese subjects.

A case-control association study was performed for 603 patients and 492 healthy controls. Because the association of *FAM167A-BLK* region with SLE is already established,[1–4] patients with RA complicated with SLE were excluded. All patients fulfilled the American College of Rheumatology classification criteria for RA.[6] The patients and the healthy controls were recruited at Matsuta Clinic, University of Tsukuba, the University of Tokyo and Juntendo University. This study was reviewed and approved by the research ethics committees of University of Tsukuba and other participating institutes. Written informed consent was obtained from all participants, except for some participants before 2001, before the enforcement of the Ethics Guidelines for Human Genome/Gene Analysis Research by the Japanese government. From such participants, oral informed consent had been obtained. In accordance with the guidelines, the latter samples were anonymised in an unlinkable fashion and were included in this study after review and approval by the ethics committee of University of Tsukuba. The genotype of rs13277113 was determined using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, California, USA).[3] Power calculation based on the risk allele frequency in the Japanese population (0.665) showed that this sample size provides 80% power to detect susceptibility genes with an allelic OR of 1.298. Deviation from Hardy-Weinberg equilibrium was observed neither in the patients nor in the controls.

A significant association with RA was replicated in the Japanese population (table 1). Although the OR was comparable to that in the Caucasian population (1.19 for rs2736340[5]), the risk allele frequency was considerably higher in the Japanese subjects than in the Caucasians (0.273 in cases vs 0.240 in controls for rs2736340[5]). The population attributable risk percentage was estimated to be 22.8% in the Japanese population and 9.3% in the Caucasian population under the dominant model. No significant difference in rs13277113 was observed between *HLA-DRB1* shared epitope positive and negative RA (data not shown).

Our observations indicate that the *FAM167A-BLK* region may be a shared genetic factor for a number of autoimmune diseases in multiple populations, but the genetic contribution may be greater in Asian populations because of the differences in the genetic background.

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Funding This work was supported by Grant-in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science (JSPS), Health and Labour Science Research Grants from the Ministry of Health, Labour and Welfare of Japan, Japan Rheumatism Foundation, The Naito Foundation and Mitsubishi Pharma Research Foundation.

Competing interests None.

Ethics approval This study was conducted with the approval of the University of Tsukuba, Juntendo University and the University of Tokyo.

Provenance and peer review Not commissioned; externally peer reviewed.

Accepted 29 August 2009
Published Online First 9 September 2009

Ann Rheum Dis 2010;**69**:936–937. doi:10.1136/ard.2009.118760

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ARTHRITIS & RHEUMATISM
Vol. 62, No. 3, March 2010, pp 890–895
DOI 10.1002/art.27303

Association of the *FAM167A–BLK* Region With Systemic Sclerosis

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Objective. **An association of single-nucleotide polymorphisms (SNPs) in the *FAM167A* (previously referred to as *C8orf13*)–*BLK* region with systemic lupus erythematosus (SLE) has been demonstrated in Caucasians and in Asians. Recent studies have shown that many genes, including *IRF5*, *STAT4*, and *PTPN22*, are shared susceptibility genes in multiple autoimmune diseases. We undertook the current study to examine whether the *FAM167A–BLK* region is also associated with susceptibility to systemic sclerosis (SSc).**

Methods. **Japanese patients with SSc (n = 309) and healthy controls (n = 769) were enrolled in a 2-tiered case–control association study. In tier 1, 124 patients and 412 controls were tested to determine association of 16 tag SNPs encompassing the *FAM167A–BLK* region with SSc. In tier 2, an additional 185 patients and 357 controls were analyzed for SNP rs13277113.**

Results. **Two haplotype blocks that correspond approximately to *FAM167A* and *BLK* were observed. In tier 1 of the study, the rs13277113A allele in the *BLK* block exhibited the most significant association with SSc after correction for multiple testing (permutated $P = 0.024$). Two SNP haplotypes formed by rs13277113 and the most significant SNP in the *FAM167A* block did not exhibit stronger association. When samples from tier 1 and tier 2 were combined, the rs13277113A allele was significantly associated with SSc (odds ratio 1.45 [95% confidence interval 1.17–1.79], $P = 6.1 \times 10^{-4}$). Association or a tendency toward association of rs13277113A with SSc was observed regardless of a patient's autoantibody profile or whether a patient had diffuse cutaneous or limited cutaneous SSc.**

Conclusion. **Our findings indicate that the rs13277113A allele is associated not only with SLE but also with SSc and that the *FAM167A–BLK* region is a common genetic risk factor for both SLE and SSc.**

Systemic sclerosis (SSc; scleroderma) is a complex disease thought to be caused by multiple genetic and environmental factors, most of which have yet to be determined. Our own recent studies, as well as studies by others, have shown that genes, such as *IRF5* and *STAT4*, that are associated with systemic lupus erythematosus (SLE) and other autoimmune diseases are also associated with the development of SSc (1–4).

Genome-wide association studies have identified an association between single-nucleotide polymorphisms (SNPs) in the *FAM167A* (previously referred to as *C8orf13*)–*BLK* region and SLE in Caucasians (5,6). We previously replicated this association in a Japanese population and also demonstrated that the genetic contribution of this SNP to SLE appeared to be substantially larger in the Japanese population than in the Caucasian population (7).

The most strongly SLE-associated SNP in this region, rs13277113, is located in the intergenic region of *FAM167A* and *BLK*, and the risk allele for SLE, rs13277113A, has been shown to be associated with low

Supported by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science, by Health and Labour Science Research Grants from the Ministry of Health, Labour, and Welfare of Japan, and by the Japan Rheumatism Foundation, the Naito Foundation, and the Mitsubishi Pharma Research Foundation.

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Submitted for publication June 14, 2009; accepted in revised form November 25, 2009.

levels of messenger RNA (mRNA) for *BLK* and high levels of mRNA for *FAM167A* (5,7). *FAM167A* encodes a ubiquitously expressed gene, the function of which remains unknown. *BLK* encodes a tyrosine kinase of the Src family, which is expressed mainly in B cells, and although the function remains to be investigated, its preferential expression in B cells suggests it plays a role in B cell signal transduction.

Autoantibodies are present in most patients with SSc, and the specificities of these autoantibodies correlate well with clinical presentations. For example, anticentromere antibodies (ACAs) are frequently found in patients with limited cutaneous SSc (lcSSc), which is rarely associated with severe internal organ damage except for pulmonary arterial hypertension. On the other hand, anti–topoisomerase I (anti–topo I) antibodies are most commonly found in patients with diffuse cutaneous SSc (dcSSc), which is often associated with internal organ damage, especially interstitial lung disease. We have previously demonstrated up-regulation of CD19 expression and decreased CD22 phosphorylation in B cells from human SSc patients (8). Furthermore, we have reported an association of *CD19* (9), *CD22* (10), and *IL10RB* SNPs (11) with SSc. These findings strongly suggest that B cell signaling dysregulation may be causally associated with SSc in a proportion of patients.

Based on these findings, we hypothesized that the *FAM167A–BLK* region may be associated not only with SLE but also with SSc. In the current study, we used a case–control association study to test our hypothesis.

PATIENTS AND METHODS

Subjects. Japanese patients with SSc (n = 309) were recruited for this study. Because the association of the *FAM167A–BLK* region with SLE has already been established (5–7), SSc patients who also had SLE were excluded. All patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for SSc (12) and were classified as having either dcSSc or lcSSc according to the classification system of LeRoy et al (13). The mean ± SD age of the SSc patients was 45.4 ± 13.9 years, and the cohort consisted of 29 men and 280 women.

Among the patients, 158 (51%) had dcSSc and 151 (49%) had lcSSc. Anti–topo I and anti–U1 small nuclear RNP (anti–U1 snRNP) levels were determined using an enzyme-linked immunosorbent assay (ELISA; Medical and Biological Laboratories, Nagoya, Japan), and specificity was confirmed by immunoprecipitation. The presence of ACAs was determined by the finding of a discrete speckled pattern on indirect immunofluorescence using HEp-2 cells and was confirmed by ELISA using recombinant human CENP-B (Medical and Biological Laboratories). One hundred patients (32%) were positive for anti–topo I antibodies, 95 (31%) for ACAs, and 22 (7%) for anti–U1 snRNP antibodies.

The control group consisted of 769 healthy unrelated Japanese subjects (359 men and 410 women). The mean ± SD age was 33.2 ± 10.3 years.

Among the cases and controls, 124 patients and 90 controls were recruited at Kanazawa University, and 185 patients and 679 controls were recruited in a collaborative effort between Tokyo Women's Medical University and the University of Tsukuba. All patients and controls were living in the central part of mainland Japan (Honshu), and the genotype distribution of rs13277113 was very similar between both groups of controls (data not shown). The study was reviewed and approved by the research ethics committees of the University of Tsukuba, Kanazawa University, and Tokyo Women's Medical University.

Genomic DNA. Genomic DNA was extracted from peripheral blood leukocytes using either a QIAamp blood kit (Qiagen, Hilden, Germany) or QuickGene (Fujifilm, Tokyo, Japan). Whole-genome amplification was performed using a GenomiPhi DNA Amplification kit according to the instructions of the manufacturer (Amersham Biosciences, Piscataway, NJ).

Genotyping. A 2-tiered association study was performed. In tier 1, 124 patients and 412 controls were screened for association of 16 tag SNPs encompassing the entire *FAM167A–BLK* region selected based on the HapMap Phase II data for Japanese ($r^2 = 0.8$; minor allele frequency ≥ 0.1). In tier 2, an additional 185 patients and 357 controls were tested for SNP rs13277113, based on the results of tier 1. Then, the results from tier 1 and tier 2 were combined and analyzed for association with susceptibility to SSc and its clinical subsets.

The TaqMan genotyping system using predesigned sequence-specific fluorescence-labeled probes (Applied Biosystems, Foster City, CA) and the ABI 7300 real-time polymerase chain reaction system (Applied Biosystems) were used for all SNPs. Using this method, genotyping was possible for all SNPs in all samples. The genotyping results for rs13277113 were confirmed by direct sequencing in 1 sample from each genotype.

Statistical analysis. Association analyses were calculated by chi-square tests using 2 × 2 contingency tables. The sample size of this study provided a detection power of 0.7 when the risk allele frequency was 0.68 (rs13277113) and the genotype relative risk was 1.3 (14). Calculation of linkage disequilibrium (LD) parameters (D′ and r^2) from the genotype of healthy controls and estimation of haplotype frequencies were performed using Haploview software, version 4.1 (Broad Institute, Cambridge, MA; online at http://www.broadinstitute.org/mpg/haploview/index.php). In tier 1, a permutation test (1,000,000 permutations) using Haploview was performed to correct for multiple testing for 16 SNPs and to test for association of SNPs and haplotypes. In the association analysis of rs13277113 with SSc and its clinical subsets, Bonferroni correction was applied. Corrected *P* values (P_{corr}) were calculated by multiplying *P* values by 6 (the number of comparisons). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were also calculated. Epistatic interaction and additive effects of *BLK*, *CD19*, and *CD22* were analyzed by logistic regression test, chi-square test, and Fisher's exact test, as

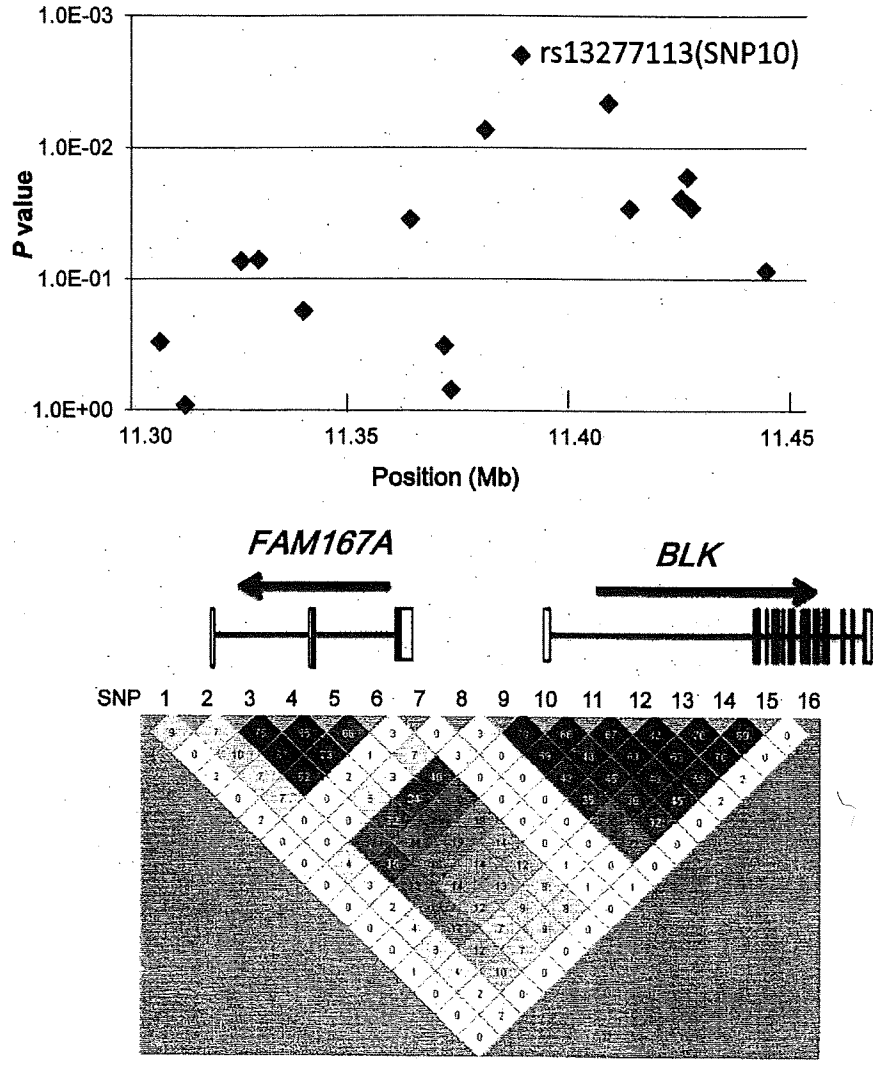

Figure 1. Allelic association of 16 tag single-nucleotide polymorphisms (SNPs) in the *FAM167A–BLK* region with systemic sclerosis, as determined in tier 1 of the association study. **Top,** *P* values for differences in allele frequencies in 124 patients and 412 controls were calculated by chi-square test using 2 × 2 contingency tables. Uncorrected *P* values for each SNP are shown. (Permutated *P* values are shown in Table 1.) The numbers of the SNPs correspond to those shown in Table 1. **Bottom,** Correlation (r^2) values based on data from 412 healthy Japanese controls were calculated using Haploview software, version 4.1. Open and solid bars indicate untranslated regions and coding regions, respectively.

recently described in a study of the role of *STAT4* and *IRF5* in the development of SSc in a European population (15).

RESULTS AND DISCUSSION

Figure 1 shows the results of the first tier of the association screening of 16 tag SNPs encompassing the *FAM167A–BLK* region in 124 Japanese patients with SSc and 412 healthy controls. An LD plot was constructed using data on the controls. Two haplotype blocks, corresponding approximately to the *FAM167A* and *BLK* genes, were observed. Eight of the 16 SNPs exhibited a tendency toward association with SSc (uncorrected $P < 0.05$), among which SNP rs13277113 in the *BLK* block showed the lowest *P* value ($P = 0.0020$).

Table 1. Association of 16 tag SNPs in the *FAM167A–BLK* region with SSc*

SNP	Correlation (r^2) with rs13277113†	Minor allele frequency: SSc patients	Minor allele frequency: Controls	*P*	Permutated *P*‡
1) rs9644737	0.0010	0.24	0.21	0.30	0.96
2) rs12548449	0.034	0.29	0.28	0.91	1
3) rs10503423	0.35	0.35	0.41	0.073	0.52
4) rs6984212	0.25	0.30	0.36	0.072	0.51
5) rs10088323	0.29	0.33	0.37	0.18	0.84
6) rs2618431	0.31	0.30	0.37	0.035	0.30
7) rs12680762	0	0.15	0.13	0.32	0.98
8) rs17799348	0.0020	0.15	0.16	0.70	1
9) rs2254891	0.78	0.21	0.30	0.0073	0.073
10) rs13277113	NA	0.24	0.34	0.0020	0.024
11) rs2736354	0.66	0.24	0.34	0.0046	0.049
12) rs2736360	0.49	0.22	0.29	0.029	0.27
13) rs1382566	0.45	0.22	0.29	0.024	0.22
14) rs11250144	0.37	0.27	0.35	0.017	0.17
15) rs12677843	0.33	0.29	0.37	0.029	0.26
16) rs2244931	0.0040	0.18	0.23	0.086	0.57

* SSc = systemic sclerosis; NA = not applicable.
† Pairwise linkage disequilibrium between rs13277113 and each of the other 15 single-nucleotide polymorphisms (SNPs) was determined by correlation (r^2) analysis of the controls.
‡ *P* values were corrected for multiple testing by permutation test (1,000,000 permutations) using Haploview software, version 4.1.

The association remained significant after correction for multiple testing by permutation test (permutated $P = 0.024$) (Table 1).

To examine the possibility that haplotypes formed by SNPs in the 2 haplotype blocks may show stronger association than either SNP alone, a permutation test was performed for the haplotypes formed by rs13277113 and the most strongly associated SNP in the *FAM167A* block, rs2618431, together with each SNP, using the "single markers and haplotypes" option in Haploview. The association observed for the single SNP rs13277113A was most significant (permutated $P = 0.0075$), rather than that observed for the haplotypes carrying this allele (for rs2618431G–rs13277113A, permutated $P = 0.025$).

To confirm the association of rs13277113, 185 additional patients and 357 controls were genotyped for rs13277113 in tier 2 of the association study. In tier 2, although allelic association did not reach statistical significance (Table 2), association was observed under the recessive model (OR 1.46 [95% CI 1.02–2.09], $P = 0.039$). Significant heterogeneity was not detected between tier 1 and tier 2 case–control sets by Breslow-Day test ($P = 0.20$). Furthermore, the allele and genotype frequencies of rs13277113 observed in healthy controls (A allele frequency 0.68; genotype frequencies AA 0.47, AG 0.43, GG 0.10) were very similar to those observed in 964 healthy Japanese whose information is part of the Genome Medicine Database of Japan (online at http://gemdbj.nibio.go.jp/dgdb/). When data from the tier 1 and tier 2 samples were combined (a total of 309 SSc patients and 769 controls), the presence of the rs13277113A allele was significantly increased in SSc patients compared with controls (OR 1.45 [95% CI 1.17–1.79], $P = 6.1 \times 10^{-4}$; $P_{corr} = 0.0037$) (Table 2). Control samples did not deviate from Hardy-Weinberg equilibrium.

Table 2. Association of rs13277113 with SSc and its clinical subsets*

Subjects (n)	Genotype, no. (%): AA	AG	GG	A allele frequency	Allelic association: OR (95% CI)	*P*†	Corrected *P*‡
Tier 1 of association study							
SSc (124)	71 (57)	47 (38)	6 (5)	0.76	1.66 (1.21–2.30)	0.0020	–
Controls (412)	179 (43)	184 (45)	49 (12)	0.66	Referent	–	–
Tier 2 of association study							
SSc (185)	108 (58)	60 (32)	17 (9)	0.75	1.26 (0.95–1.67)	0.11	–
Controls (357)	175 (49)	150 (42)	32 (9)	0.70	Referent	–	–
Total SSc patients (309)	179 (58)	107 (35)	23 (7)	0.75	1.45 (1.17–1.79)	6.1×10^{-4}	0.0037
dcSSc (158)	93 (59)	54 (34)	11 (7)	0.76	1.50 (1.14–1.98)	0.0041	0.025
lcSSc (151)	86 (57)	53 (35)	12 (8)	0.75	1.39 (1.05–1.84)	0.021	0.13
Anti–topo I–positive (100)	60 (60)	32 (32)	8 (8)	0.76	1.51 (1.07–2.12)	0.018	0.11
ACA-positive (95)	60 (63)	31 (33)	4 (4)	0.79	1.84 (1.28–2.65)	9.8×10^{-4}	0.0059
Anti–U1 snRNP–positive (22)	16 (73)	5 (23)	1 (5)	0.84	2.52 (1.14–5.53)	0.022	0.13
Total controls (769)	354 (46)	334 (43)	81 (11)	0.68	Referent	–	–

* Significant heterogeneity was not observed between the tier 1 and tier 2 case–control sets, as determined by Breslow-Day test ($P = 0.200$). SSc = systemic sclerosis; OR = odds ratio; 95% CI = 95% confidence interval; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; anti–topo I = anti–topoisomerase I; ACA = anticentromere antibody; anti–U1 snRNP = anti–U1 small nuclear RNP.
† *P* values for the differences in allele frequencies were calculated by chi-square analysis using 2 × 2 contingency tables.
‡ Bonferroni correction was applied by multiplying the *P* values by 6 (the number of comparisons).

We next investigated whether the association was limited to particular clinical characteristics of SSc, such as a patient's autoantibody profile and whether a patient had dcSSc or lcSSc. As shown in Table 2, when compared with healthy controls, a tendency toward association was detected regardless of the clinical subset being tested, among which association of rs13277113 with dcSSc (P_{corr} = 0.025) and ACA positivity (P_{corr} = 0.0059) remained significant after Bonferroni correction. The cutaneous disease subsets and autoantibody profiles may appear contradictory. Only ~60% of the patients with dcSSc and lcSSc were positive for anti–topo I antibodies and ACAs, respectively. The apparent discrepancy was likely caused by relative enrichment of the risk allele in anti–topo I–negative dcSSc patients but not in ACA-negative lcSSc patients (data not shown). It should also be noted that such a difference in statistical significance was influenced by a difference in the sample size of each subset, because the risk allele frequencies and ORs were not substantially different. Of interest, although statistical association was not attained due to the small number of the patients, the subset of patients who were anti–U1 snRNP–positive exhibited the highest OR. This issue needs to be investigated in future studies with larger sample sizes.

Because the sex ratio was substantially different between patients and controls, the association of rs13277113A with SSc was also examined using logistic regression analysis by including sex as a parameter. The association of rs13277113 remained significant even after adjusting for sex (OR 1.50 [95% CI 1.29–1.73], P = 0.0055 under the recessive model). Although the controls were younger than the patients, in view of the low incidence of SSc, it is highly unlikely that a substantial number of the controls would later develop SSc. Even in such a case, our present data would be considered to be conservative and cannot be a cause of a Type I error.

We previously reported an association of SNPs in the B cell signaling molecules such as *CD19* and *CD22* with SLE in a Japanese population (9,10). The genotype frequency of *BLK* rs13277113 in patients with and those without the risk genotype of *CD19* (rs73533858T carriage) or *CD22* (rs34826052A/A) was not significantly different (P = 0.95 and P = 0.77, respectively, by Fisher's exact test). In addition, a case–control analysis showed that the association of *BLK* was similarly observed in patients who did and those who did not carry the *CD19* risk genotype. Because the *CD22* risk genotype was absent in the genotyped controls, such an analysis could not be performed. Thus, no evidence for epistatic interaction was detected.

We next tested whether the risk alleles of *BLK*, *CD19*, and *CD22* have an additive effect. When the association between the number of risk alleles in the 3 genes in each individual and the risk of developing SSc was examined using logistic regression analysis, the OR was shown to increase 1.55-fold for each addition of a risk allele (P = 0.0035). When compared with individuals with 0 or 1 risk allele, the OR (95% CI) was 1.80 (0.89–3.66) in individuals with 2 risk alleles, 2.53 (1.14–5.58) in individuals with 3 risk alleles, and 4.53 (1.44–14.27) in individuals with 4 or 5 risk alleles, respectively. Thus, these risk alleles appeared to have an additive effect in conferring susceptibility to SSc.

This report is the first to describe the association of a *FAM167A–BLK* region polymorphism with genetic susceptibility to SSc. The SNP rs13277113 is the same SNP that accounts for the genetic effect of the *FAM167A–BLK* region in SLE (5,7). Recently, many genes, including *IRF5*, *STAT4*, and *PTPN22*, have been shown to be associated with multiple autoimmune diseases. The findings of the current study suggest that the *FAM167A–BLK* region may also contribute to a genetic background shared by multiple autoimmune diseases. Further studies of SSc and other autoimmune diseases are required to test this hypothesis.

The SNP rs13277113 is located in the intergenic region between *FAM167A* and *BLK*. Thus far, molecular mechanisms that link this SNP and autoimmunity have not been reported. The risk allele has been reported to be associated with lower expression of *BLK* and higher expression of *FAM167A* (5,7); however, at this point, the direct functional role of this SNP is unclear, and it is possible that this SNP represents a proxy of other functional polymorphisms causally related to the pathogenesis. Full resequencing of the *FAM167A–BLK* region will address such a question.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Tsuchiya had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ito, Kawasaki, Tsuchiya.

Acquisition of data. Ito, Kawaguchi, Kawasaki, Hasegawa, Kawamoto, Fujimoto, Takehara, Sato, Hara, Tsuchiya.

Analysis and interpretation of data. Ito, Kawasaki, Ohashi, Tsuchiya.

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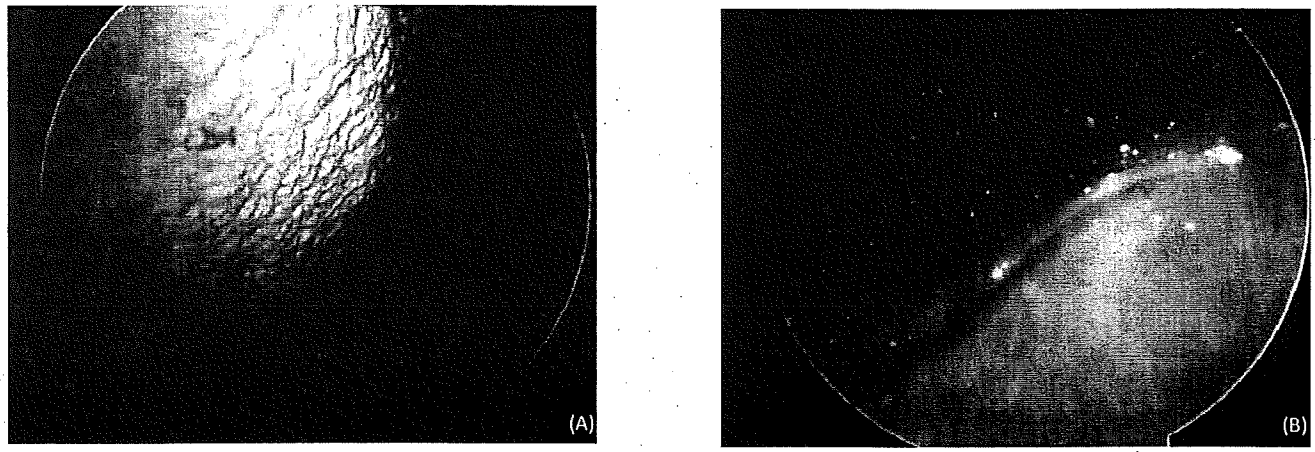
DOI 10.1002/art.27301

Clinical Images: Gout revealed on arthroscopy after minor injury



The patient, a 34-year-old man who had sustained a minor, sports-related twisting injury, presented with significant pain in the lateral area of the left knee; tenderness was most prominent medially. Magnetic resonance imaging results suggested a small lateral meniscal tear, and arthroscopy was performed. Widespread chalky plaques were noted on cartilaginous surfaces (**A**), while the synovium had a "snow-speckled" appearance from the deposits (**B**). Even a minor injury can trigger an attack of gout in major joints, which can mimic other conditions.

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