肘関節滑膜切除術

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はじめに

関節リウマチ(以下，RA)肘に対する滑膜切除術は，その臨床的効果が認められている術式の1つである。施術にあたっては滑膜切除が完全に近い形で行われることが望まれる。本稿では，後側方進入法を用いた肘関節滑膜切除術を紹介する。

手術適応

われわれのもの[3]も含めて過去の多くの報告[1),6)]では，比較的進行期以降の症例に対する手術結果が報告されている。それによると，進行期のRAでも除痛効果は認められており，適応は広いといえる。最近われわれが行ったretrospective study[4]では，よい結果が期待できる対象は，術前の日整会肘疾患判定基準の点数には関係なく，内科的にRAがコントロールされており，X線学的に破壊が進行していない症例であることが判明した。一方，ムチランス型RAでは骨破壊の進行が早く，適応がないことが判明した。

術前準備

RAのコントロール

RAの内科的コントロールは，滑膜切除術の成果を維持するのに重要である。今までの著者の経験から，内科的コントロールがよくできている例では，術後成績も好結果を維持されていることがわかってきた[5]。術前にできる限りRAの病勢をコントロールすることが大切であることは論を待たないが，術後も内科的治療の継続が大切であることを教育しておく。

手術機器の準備

滑膜切除術に特殊な機器は必要としない。一般的手術機器として，エアトーム，大小のリウエル，骨膜剥離子(ラスパトリウム)，電動あるいは手動ドリルなどが必要である。

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手術体位

手術体位は仰臥位で患肢は胸上位とする。肩後方に砂嚢を敷いて内分回しをしやすくする。手術台の高さを術者が操作をしやすい高さに調整する。

高齢者のRA患者では，骨粗鬆症から生じる円背のため完全な仰臥位をとることが難しいことがある。そのような場合は患肢側を浮かす亜側臥位とし，手術台にはエアーマットなどを敷いて，患者の腰背部痛を予防するとよい。

手術手技

駆血

上肢の正確な手術には駆血が必要である。駆血帯は清潔空気駆血帯あるいはEsmarch駆血帯を使用する。前者を使用すれば空気圧250～280mmHgで90分程度は安全に駆血下手術が可能である。後者を使用する場合は駆血帯をきつく巻きすぎると駆血圧が上がり駆血帯麻痺を起こすことがある。これは駆血時間に関係なく生じるので注意する。駆血帯はできる限り中枢に装用し，皮切の邪魔にならないようにする。

皮切

術者は肘関節を90°に屈曲して肘の後方が展望できる位置に立つ。

皮切は肘頭レベルより約7～8cm中枢上腕外側に始まり，肘頭より4～5cm末梢尺骨稜に終わる。中枢側では上腕骨外側筋間中隔より後方を長軸に沿って入り，肘関節レベルで後方に向かい，尺骨後方の稜線で停止する(図1)。最近では尺側の処置をしやすくするため，より後方よりの皮切を選択することが多い。

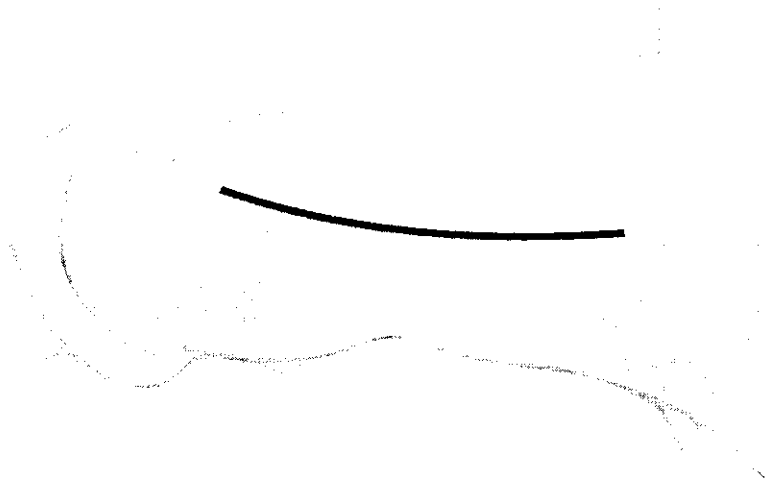

図1　皮切
肘頭より上腕後外側，約7～8cm中枢に始まり，4～5cm末梢尺骨上に終わる。右上肢を頭側から鳥瞰したもので，左は末梢，下は橈側。

尺骨神経の同定，保護(図2)

皮下組織を内側に剥離して，上腕骨内顆やや後方を走る尺骨神経を同定する。栄養血管をなるべく傷つけないように注意しながら，尺骨神経を肘部管の中枢でテープをかけて保護する。この際，尺骨神経の扱いが粗暴であると，術後不快なしびれが続くこととなるので極力ていねいに扱う。術前に肘部管症候群を合併している例では，肘部管開放術と内側上顆切除術を追加する。関節破壊が強いために尺骨神経麻痺をきたしている例では，前方移動術が必要なので，尺骨神経の中枢，末梢側へのさらなる剥離が必要となる。

肘関節の露出および滑膜の切除

外側では，上腕三頭筋と腕橈骨筋間の外側上腕筋間中隔を骨膜に向かって鋭的に進入する。この際，筋間中隔には血管が多いので，電気凝固器を用いてていねいな止血操作を行う。上腕骨に達するとラスパトリウムを用いて骨膜下に前方，後方へ剥離を進める。後方では後方関節包を同定し，その周辺を十分剥離する。前方では上腕筋，腕橈骨筋，手根伸筋の下，骨膜下に前方関節包を露出する。これらの関節包は滑膜で膨隆している。末梢への切開は，上腕骨外顆をすぎて肘筋の線維走行に沿って尺骨後縁に至る。肘筋と三頭筋筋膜の尺骨付着部では，これらの連続性を不用意に断裂させることのないようにていねいに骨膜下剥離を進める。

この操作までで橈側側副靱帯の外観が把握できる。靱帯の前方，後方の関節包，滑膜を切除し，靱帯幅を決定する。橈側側副靱帯は遠位に移行する輪状靱帯とは明確な区別ができないが，後の一次縫合に備えた縫い代を残し，高さ2cmのZ字状の切開を入れて靱帯を切離する(図3)。

内反を加えると腕橈関節が直視できる。ここで同部の滑膜切除を行うが，橈骨頭の周辺は滑膜炎が強いのが一般的で，輪状靱帯を反転し，十分滑膜切除を行う。橈骨頭の切除を支持する報告[2]も多いが，切除しなくても滑膜切除は可能で著者は

図2　尺骨神経の保護
術中に尺骨神経への損傷を避けるため，必ずテープで保護しておく。右上肢を真上から鳥瞰したもので，左は末梢，下は橈側。

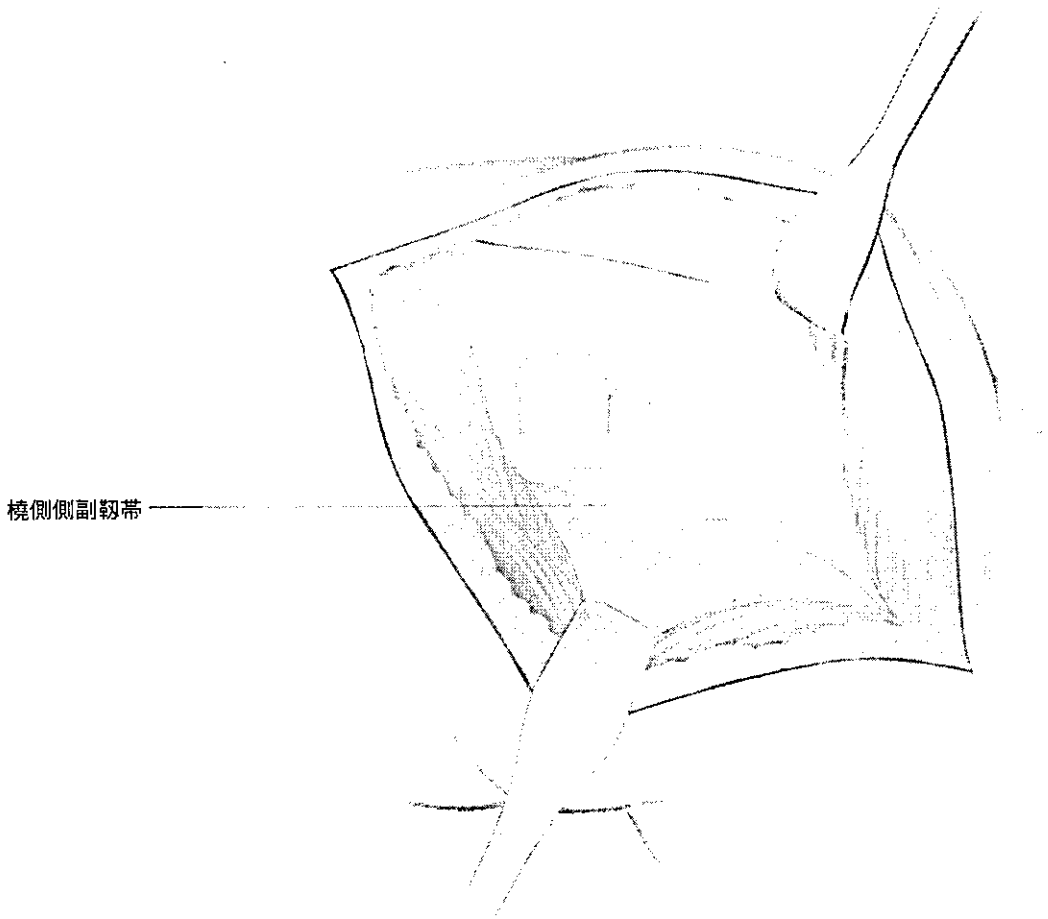

図3　橈側側副靱帯の切離
あとの縫合に備えて、Z字状に切離する。右上肢を頭側から鳥瞰したもの。

温存している。滑膜切除はリウエル，メスを適宜用いて行う。

後方では，上腕三頭筋の肘頭付着部を外側から内側に向かって骨膜下に剥離する。同部は菲薄であるので剥離には細心の注意が必要である。これには鋭利なメスあるいはラスパトリウムを適宜使い分けて進める(**図4**)。剥離の方向を誤らなければメスを用いたほうが容易である。一般に，RA肘での三頭筋筋膜の剥離は難しく，また完全な剥離は必ずしも必要ではない。内側の滑液膜炎の状態をよく観察して，必要にして十分な剥離を行えばよい。上腕三頭筋を内側に牽引すると後方の滑膜切除が可能となる。

上腕三頭筋を尺側に避けながら肘を内反していくと，腕橈関節から順次，内側へ視野が開けてくる。関節前方では筋群を筋鉤で牽引すれば，肘関節内側の滑膜切除が可能である。比較的早期RA症例で関節に弛みがない，あるいは上腕三頭筋肘頭付着部を温存したい例では，尺骨神経をテープで保護し，三頭筋の後内側，尺側側副靱帯の後方部分を縦切し，腕尺関節へ直接進入，ここから内側の滑膜切

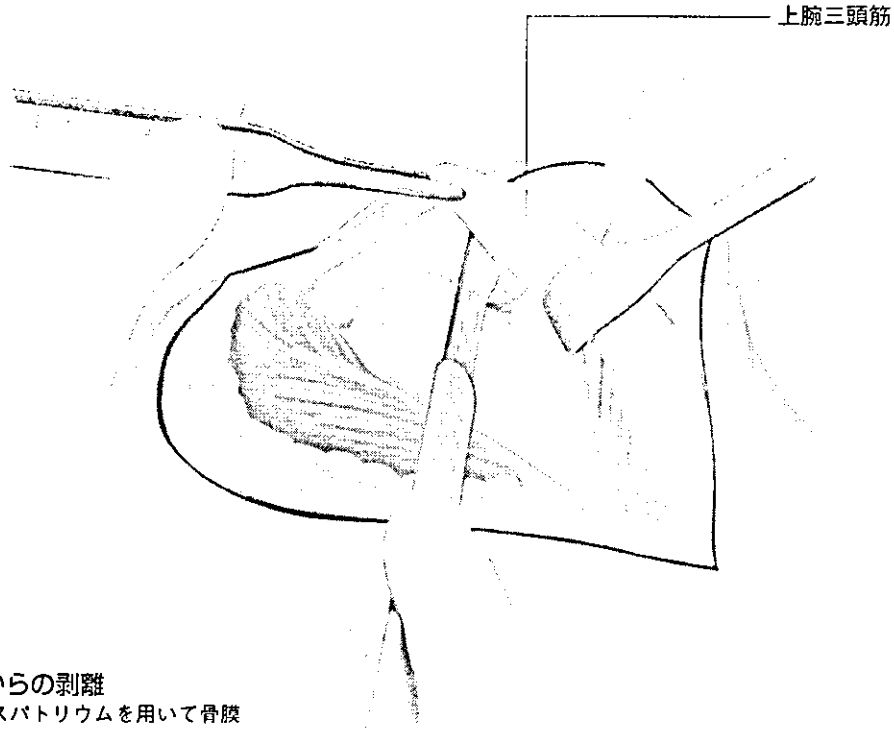

図4　上腕三頭筋膜の肘頭からの剥離
これには鋭利なメスあるいはラスパトリウムを用いて骨膜下にていねいに剥離する。右上肢を頭側から鳥瞰したもの。

除を行う(図5)。

最後に全体を見わたし，軟骨下層に浸潤するパンヌスがあれば，同部の滑膜もできる限り切除するように努める。

創閉鎖

創閉鎖に先立ち，肘頭に縫合糸を通すドリル穴(直径2～3mm)を2穴準備する(図6)。十分の洗浄ののち，吸収性局所コラーゲン止血剤(インテグラン®など)シートを，出血の予想される部位に留置する。駆血帯を解除し，およそ5～7分圧迫止血する。止血シートを除去し，血管性出血がないことを確認ののち，非吸収性の縫合糸2本で上腕三頭筋膜を先ほど準備した肘頭穴にやや緊張をもって縫着する。橈側側副靱帯も非吸収糸にて一次的に縫合する。持続吸引ドレーンを肘関節前面に留置し，剥離した上腕筋群を可及的に一次縫合する。

創縫合ののち，上腕ギプスシーネで肘をおよそ90°屈曲位固定する。

後療法

術後24～48時間でドレーンを抜去する。

術後3日目から昼間はギプスシーネを除去し，自動可動域訓練を行う。夜間ギプスシーネは術後3週間装用する。術後3週間で可動域の回復が悪い場合には，他動可動域訓練を追加する。術後6～8週でほぼ術前まで回復する。

なお，可動域回復補助のためのCPM(continuous passive motion)は，多関節罹患

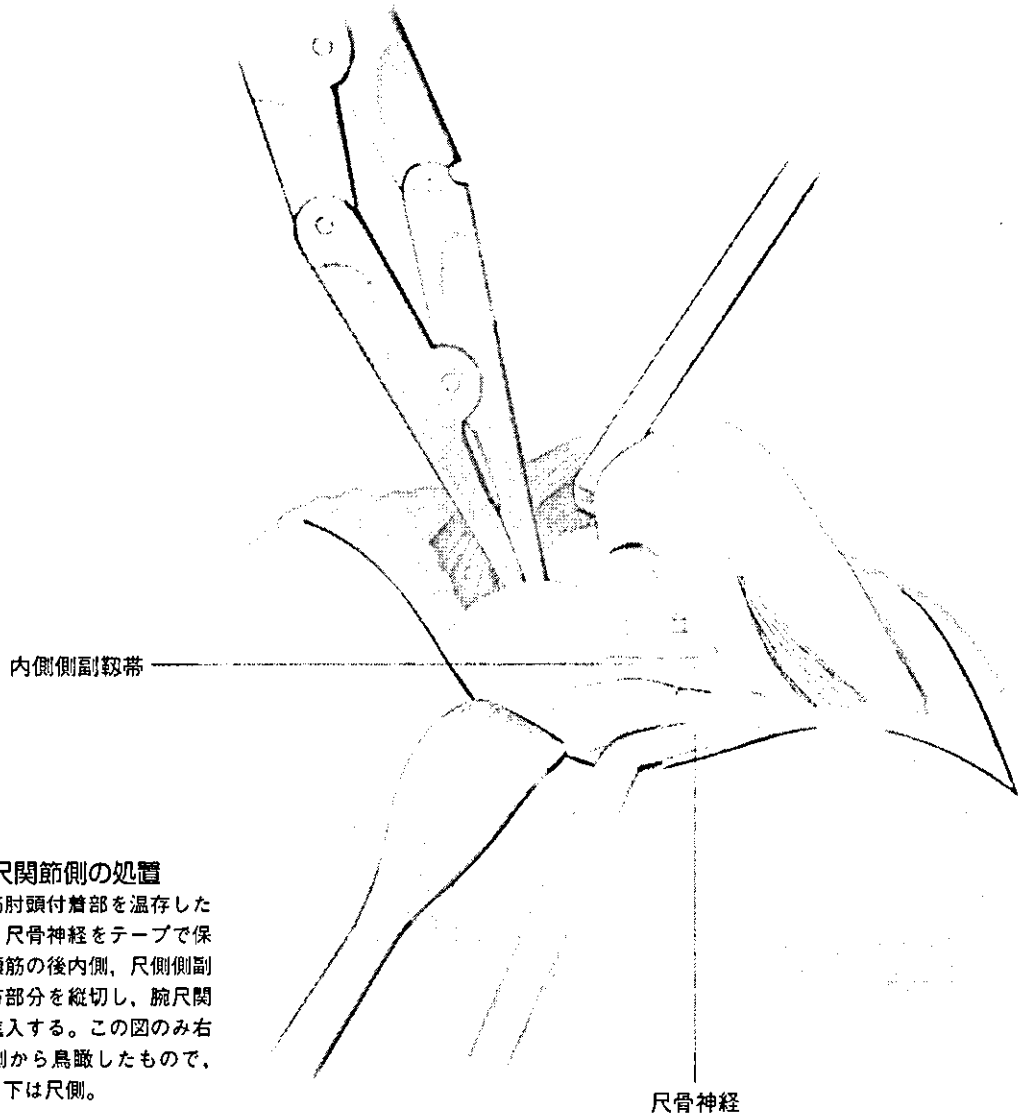

図5　腕尺関節側の処置
上腕三頭筋肘頭付着部を温存したい場合は、尺骨神経をテープで保護し、三頭筋の後内側，尺側側副靱帯の後方部分を縦切し，腕尺関節へ直接進入する。この図のみ右上肢を尾側から鳥瞰したもので，左は中枢，下は尺側。

RAの患者には装用が煩雑でさらに苦しみを与えること，可動域の回復が肘関節症術後に比べて容易なこと，などの理由から，現在では基本的に用いていない。しかし，痛みあるいは筋力不足のために訓練が軌道に乗らない症例では昼間4～6時間装用することもある。

合併症の防止対策

人工肘関節形成術に比べ合併症の頻度は低いが，考えられる合併症の防止対策を列挙する。

肘伸展力低下

肘頭部への上腕三頭筋Sharpy線維が断裂されたときに問題となる。これを防ぐ対策としては，上腕三頭筋の肘頭部からの剥離を外側最小限に抑え，連続性を保

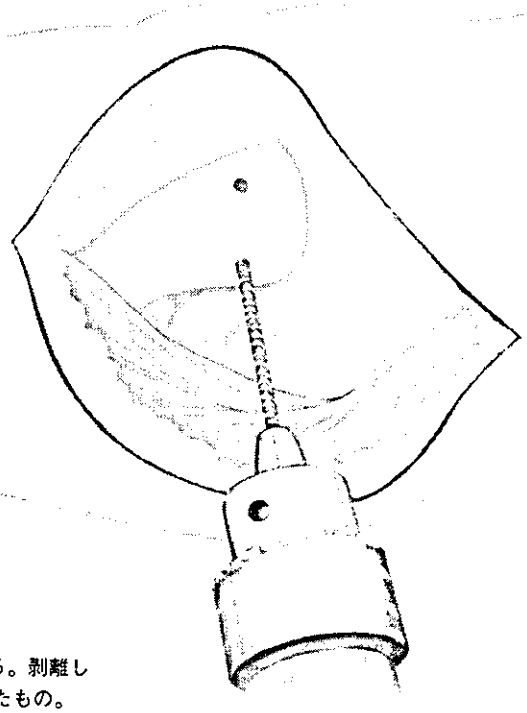

図6　上腕三頭筋膜縫着用のドリル穴の削孔
肘頭に非吸収糸の通る2～3mm直径の穴を2つ作製する。剥離した上腕三頭筋膜を縫着する。右上肢を真上から鳥瞰したもの。

つことにある。不幸にして断裂された場合には肘頭に骨孔を作製し，ここを通した長掌筋腱で三頭筋を編み上げ縫合する。

亜脱臼

術前から不安定性が強い肘で，腕橈関節の脱臼が起こることがある。予防には外側側副靱帯の一次縫合を正確に行うことが重要である。

感染

感染の原因は不必要に長い手術時間，血腫の形成，洗浄の不足などがあげられる。予防には，手際のよい手術，正確な止血操作および持続吸引ドレーンの留置，十分な洗浄が求められる。

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MP関節

RA手指伸展障害をきたしたMP関節伸筋腱脱臼例の検討

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Finger Extension Disturbance Caused by Dislocation of the Extensor Tendon in Patients with Rheumatoid Arthritis

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Extensor dislocations at the metacarpophalangeal (MP) joint result in ulnar drift and/or extension disturbance of the fingers in rheumatoid arthritis (RA). Extension disturbance of the fingers was rarely encountered in spite of ulnar drift of the fingers. In such cases, it is often misdiagnosed as extensor ruptures at the wrist level. We have experienced six such cases who had developed finger extension disturbance caused by dislocation of the extensors.

Nineteen fingers of six cases were included in this study. Five of them had RA, and the one systemic lupus erythematodes (SLE). The affected fingers were two-index, five-middle, seven-ring and five-little fingers. For reconstruction, central relocation of the extensor tendon and division of the ulnar jucturae tendinum were performed, and the abductor digiti minimi was divided when little finger was involved. Tenolysis of the extensor at the wrist was combined in two cases showing adhesion, and synovectomy of the MP joint and the extensor were combined in four fingers, and oblique retinacular ligament reconstruction was combined in six fingers with swan neck deformity.

Active ROM (extension/flexion) of the MP joints improved from −65/87° before operation to −5/87° at follow-up in the 3rd MP joint, from −50/83° to −17/82° in the 4th MP joint, and from −73/85° to −18/83° in the 5th MP joint respectively.

Extension disturbance caused by the dislocation of the extensor tendons can be corrected by the conventional central relocation technique if the MP joints are fairly preserved.

はじめに

関節リウマチ（以下，RA）手におけるMP関節レベルでの伸筋腱脱臼は指の尺側偏位や伸展不能の原因となるとされている[5]．前者は時に遭遇するが，後者に遭遇することは比較的まれで，伸筋腱断裂と診断を誤ることがある．我々は過去に指伸展障害を主訴とし，再建術を行った本症を6例経験した．これらについて成績を報告し診断上の問題点について検討した．

対象

伸筋腱脱臼によって手指の伸展困難をきたした6例19指を対象とした．症例の内訳はRA 5例，全身性エリテマトーデス（以下，SLE）1例，男性2例，女性4例，年齢は51～75歳（平均61歳）であった．罹患指は示指：2，中指：5，環指：7，小指：5指であった．

RAの5例ではすべて指伸展不能が，SLEでは手指の伸展困難，弾発現象（trigger phenomenon）が主訴で

Key words：rheumatoid arthritis（関節リウマチ），extensor dislocation（伸筋腱脱臼），finger extension disturbance（指伸展障害），metacarpophalangeal joint（中手指節間関節），central relocation（中央移転）

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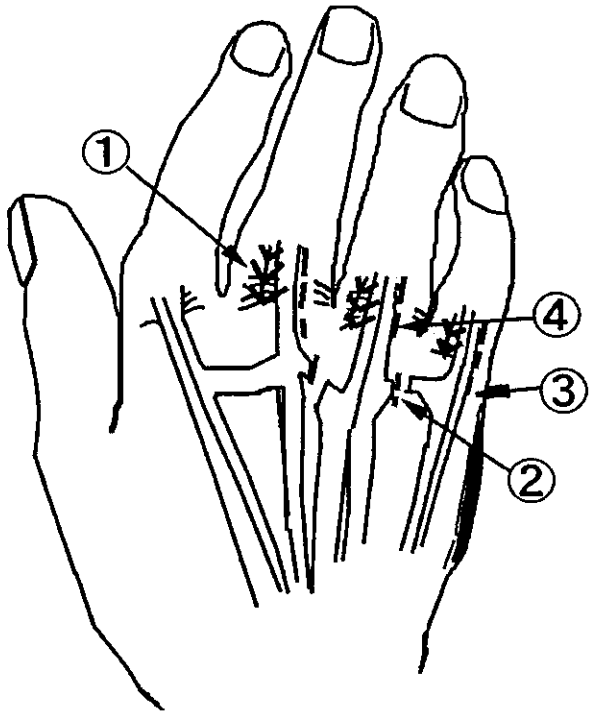

Fig. 1 Operation
①Radial sagittal band is incised longitudinally and plicated to relocate the extensor tendon.
②Juncturae tendinum are divided.
③The abductor digiti minimi is divided at its insertion to the basal phalanx.
④Ulnar sagittal band is incised if necessary.

あった．過去に手関節での腱再建術を受けた例が2例あった．MP関節の破壊，脱臼の著しいものは手術の適応外とした．

手 術 方 法

再建術として指伸筋腱の橈側指背腱膜を縦切し，尺側に脱臼した伸筋腱を整復し橈側指背腱膜を重ねて縫うことにより縫縮し，尺側隣接指腱間結合を切離するcentral relocation術[6]を全例に施行した．整復が十分に得られない場合には伸筋腱の尺側指背腱膜の縦切を追加した．小指罹患例では小指外転筋切離術を追加した（Fig. 1）．

併用手術として，手関節での伸筋腱癒着2例では腱剝離を，MP関節滑膜炎，伸筋腱滑膜炎を併発していた1例4指には滑膜切除術を，swan neck変形の1例6指ではLittlerの斜支靱帯再建術[4]を追加した．

結 果

術前診断で手関節レベルでの伸筋腱断裂とした誤診が1例あった．この例では術中伸筋腱が無傷であることを確認し，引き続き中環小指のMP関節レベルでの伸展機構を観察したところ，伸筋腱の尺側脱臼を認めたのでこれらのcentral relocation術と小指外転筋切離術を追加した．

RA 5例の術前後のMP関節自動可動域の変化は症例数が5指以上あった中環小指について，中指：−65/87°（平均自動伸展/平均自動屈曲，以下，同様）から−5/87°へ，環指：−50/83°から−17/82°へ，小指：−73/85°から−18/83°へ改善していた．

SLE例では術前からMP可動域制限はなく，屈曲位から指伸展開始に伸展補助を要するいわゆる弾発現象が主訴であったが術後は円滑な伸展運動が可能となった．

成績不良例が1例あった．該当例の術前後の自動可動域は示指：−40/80°（伸展/屈曲，以下，同様）から0/80°へ，中指：−50/80°から0/80°へ，環指：−60/85°から−55/90°へ，小指：−90/100°から−55/90°と変化していたが，環小指で伸展域の回復が不良であった．

成 績 不 良 例

75歳，男性．1997年にRA発症．指伸展不能を主訴に来院した．MP関節滑膜炎と伸筋腱滑膜炎が著明であった．2002年7月，手関節での伸筋腱滑膜切除術，第2-5 MP関節滑膜切除術および同部伸筋腱central relocation術を施行した．術後，再建伸筋腱を用手的に牽引したが，MPの伸展は不十分であった．

術後6ヵ月時点では指伸展は示中指については改善しているものの環小指についてはほとんど改善していない（Fig. 2）．

成 績 良 好 例

56歳，女性．1989年にRA発症．半年前から指の尺側偏位と環小指の伸展が次第に困難となり受診した．1997年6月に手術を行った．環小指の伸筋腱は尺側に脱臼していた．これを元位置に復し，小指固有外転筋を切離した．術後3年現在，指の屈伸は回復している．尺側偏位は術前に比べ矯正されている（Fig. 3）．

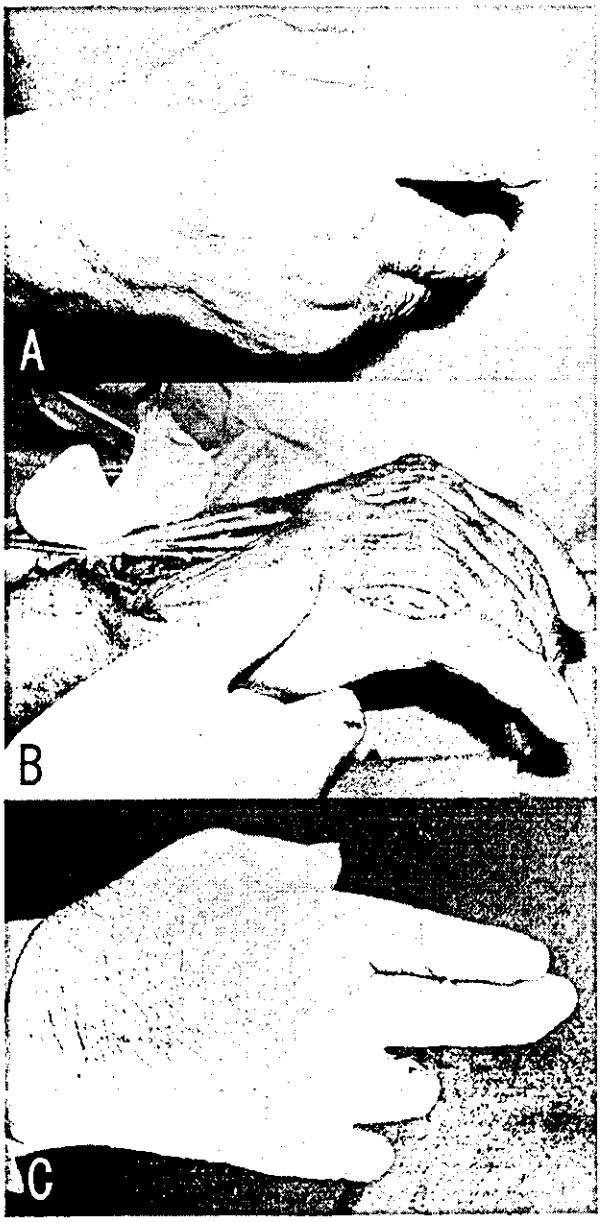

Fig. 2 Illustrative case of a poor result
A : Preoperative extension of fingers.
B : After extensor tenosynovectomy, synovectomy of the 2-5th MP joints and central relocation of the extensor tendons. Note extension lag even with manual distraction of extensor.
C : Extension of fingers six months after operation. Marked extension lag was left in the ring and little fingers.

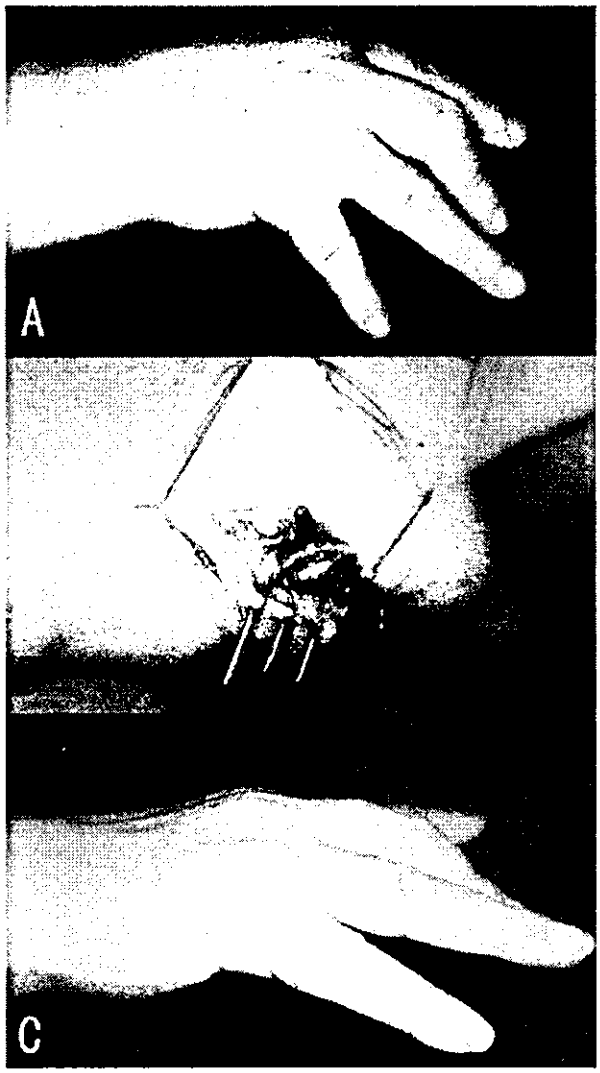

Fig. 3 Illustrative case of a good result
A : Preoperative extension of the fingers. Extension lag and ulnar drift was noted in the ring and little fingers.
B : At operation, the abductor digiti minimi was divided and central relocation of the extensor tendons was performed.
C : Three years after operation, full extension of the ring and little finger was maintained, and ulnar drift was corrected.

考　　察

RA の MP 関節における伸筋腱の尺側脱臼は MP 滑膜炎の増悪による矢状索のゆるみとともに緩除に進行し，本来 MP 関節伸展力として働くべき伸筋腱は尺側偏位を増悪する力となり，最終的には MP 関節の回転

中心より掌側を通過し MP 関節伸展不能[5]を招来することとなる.

病変が MP 関節に限局される疾患では診断は困難でないが, RA のように手関節も同時に罹患しているような例では, 臨床像は手関節レベルでの伸筋腱断裂に酷似し診断を誤ることがある. 指尺側偏位を伴う伸展不能例では MP 関節での伸筋腱脱臼を鑑別診断に入れる必要がある.

SLE あるいは健常者では本症は MP 関節伸展を補助してやればある段階で伸展可能となる trigger phenomenon[1]を呈するので本症の診断は可能である. RA ではこの現象はまれである. 最も信頼できる診断方法は MP 関節最大屈曲時に浮き彫りにされる伸筋腱が尺側へ脱臼していることを確認することである.

再建法として伸筋腱尺側を半裁して橈側へまわす方法[2], loop を作製する方法[3], 伸筋腱を中央へ再度移転する central relocation 法, そして基節骨基部へ縫着する方法などが知られている. MP 関節面の比較的保たれた症例では我々の利用した伸筋腱の central relocation により良好な結果が期待できると思われる. しかし, MP 関節が破壊されたり, 脱臼をきたしている場合には伸筋腱に伝わるベクトルが矯正されても円滑な伸展は望むべくもない. 今回の対象症例で成績不良例では MP 関節の破壊, 脱臼は軽度であったが, 併用した手関節伸筋腱滑膜切除後に伸筋腱の緩みが確認された.

ま と め

1. MP 関節レベルの伸筋腱脱臼によって指伸展困難をきたした RA 性疾患 6 例 19 指についてまとめた.
2. その臨床像は時に手関節での伸筋腱断裂と酷似するので本疾患との鑑別を要した.
3. 手術治療として MP 関節が比較的保たれた例では伸筋腱の central relocation 術が有効であった. しかし, 伸筋腱にゆるみがあった例では成績は不良であった.

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Functional haplotypes of *PADI4*, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis

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Individuals with rheumatoid arthritis frequently have autoantibodies to citrullinated peptides, suggesting the involvement of the peptidylarginine deiminases citrullinating enzymes (encoded by *PADI* genes) in rheumatoid arthritis. Previous linkage studies have shown that a susceptibility locus for rheumatoid arthritis includes four *PADI* genes but did not establish which *PADI* gene confers susceptibility to rheumatoid arthritis. We used a case-control linkage disequilibrium study to show that *PADI* type 4 is a susceptibility locus for rheumatoid arthritis ($P = 0.000008$). *PADI4* was expressed in hematological and rheumatoid arthritis synovial tissues. We also identified a haplotype of *PADI4* associated with susceptibility to rheumatoid arthritis that affected stability of transcripts and was associated with levels of antibody to citrullinated peptide in sera from individuals with rheumatoid arthritis. Our results imply that the *PADI4* haplotype associated with susceptibility to rheumatoid arthritis increases production of citrullinated peptides acting as autoantigens, resulting in heightened risk of developing the disease.

Rheumatoid arthritis is one of the most common human systemic autoimmune diseases. It is characterized by inflammation of synovial tissues and the formation of rheumatoid pannus, which is capable of eroding adjacent cartilage and bone and causing subsequent joint destruction. Previous studies have indicated that risk of the disease in siblings of affected individuals (λ_{sib}) is 2–17 times higher, suggesting the importance of genetic factors in rheumatoid arthritis[1]. Multiple genes are believed to contribute to rheumatoid arthritis susceptibility, but the only locus that has been conclusively associated with the condition is the *HLA-DRB* locus, which accounts for about one third of the genetic component[2–4]. Recently, four sibling-pair linkage studies from Europe, North America and Japan were published[5–8]. Although no common loci apart from the *HLA* region were suggested by all the studies, some were suggested by multiple studies. Chromosome 1p36 represents one such locus. Cornelis *et al.*[5] reported an association between rheumatoid arthritis and *D1S228* that identified nucleotides 363,575–363,702 on NT_004873.12 in a study using 114 sibling pairs ($P = 0.0065$). Shiozawa *et al.*[8] obtained a single-point lod score of 3.58 at *D1S214* that identified nucleotides 1,089,077–108,972 on NT_028054.9 and also observed lod scores of 3.77 as a single-point analysis and 6.13 as a multi-point analysis at *D1S253* that identified a region 1.5 cM telomeric from *D1S214* (located in GB4 map by the International RH Mapping Consortium but not annotated in the Reference Sequence of genomic DNA by NCBI), using 41 families. *D1S228* and *D1S214* are located 6.7 Mb apart according to the Reference Sequence build 33 from the National Center for Biotechnology Information.

The gene region located 3.1 Mb and 9.8 Mb centromeric from *D1S228* and *D1S214*, respectively, contains clusters of enzymes that are functionally associated with the production of rheumatoid arthritis–specific autoantibodies. These enzymes are the peptidylarginine deiminases (PADIs), which posttranslationally convert arginine residues to citrulline. Citrullinated epitopes involved in a peptidic link are the most specific targets of rheumatoid arthritis–specific autoantibodies. Citrullination is related to two rheumatoid arthritis–specific autoantibody systems: those directed against perinuclear factor/keratin and against Sa[9,10]. Assays of antibodies to citrullinated peptide can

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be used as valuable diagnostic tools[11,12]. The clinical importance of measuring antibodies to citrullinated peptide and the specificity of autoantibodies suggests a specific role of citrullination and PADIs in the pathophysiology of rheumatoid arthritis. In addition, the appearance of antibodies to citrullinated peptide in sera from affected individuals in the very early phase of disease manifestation implies that citrullination is involved in the triggering phase or the acute phase of the disease[13]. The presence of citrullinated peptides in rheumatoid arthritis synovial tissue has also been reported, suggesting the involvement of PADIs in the pathomechanisms of rheumatoid arthritis[14–16].

We carried out a case-control association study using single-nucleotide polymorphisms (SNPs) discovered by the Japanese Millennium Genome Project in the 1p36 region containing the genes *PADI1*, *PADI2*, *PADI3* and *PADI4*. This study identified a haplotype associated with susceptibility to rheumatoid arthritis in *PADI4* but not in neighboring *PADI* genes. We confirmed that *PADI4* was expressed in hematological cells by northern-blot hybridization and in synovial tissue of individuals with rheumatoid arthritis by *in situ* RT–PCR and immunohistochemistry. Moreover, the susceptibility haplotype of *PADI4* was related to levels of antibody to citrullinated filaggrin in sera of individuals with rheumatoid arthritis. We also identified a difference in mRNA stability between non-susceptibility and susceptibility variants of *PADI4*.

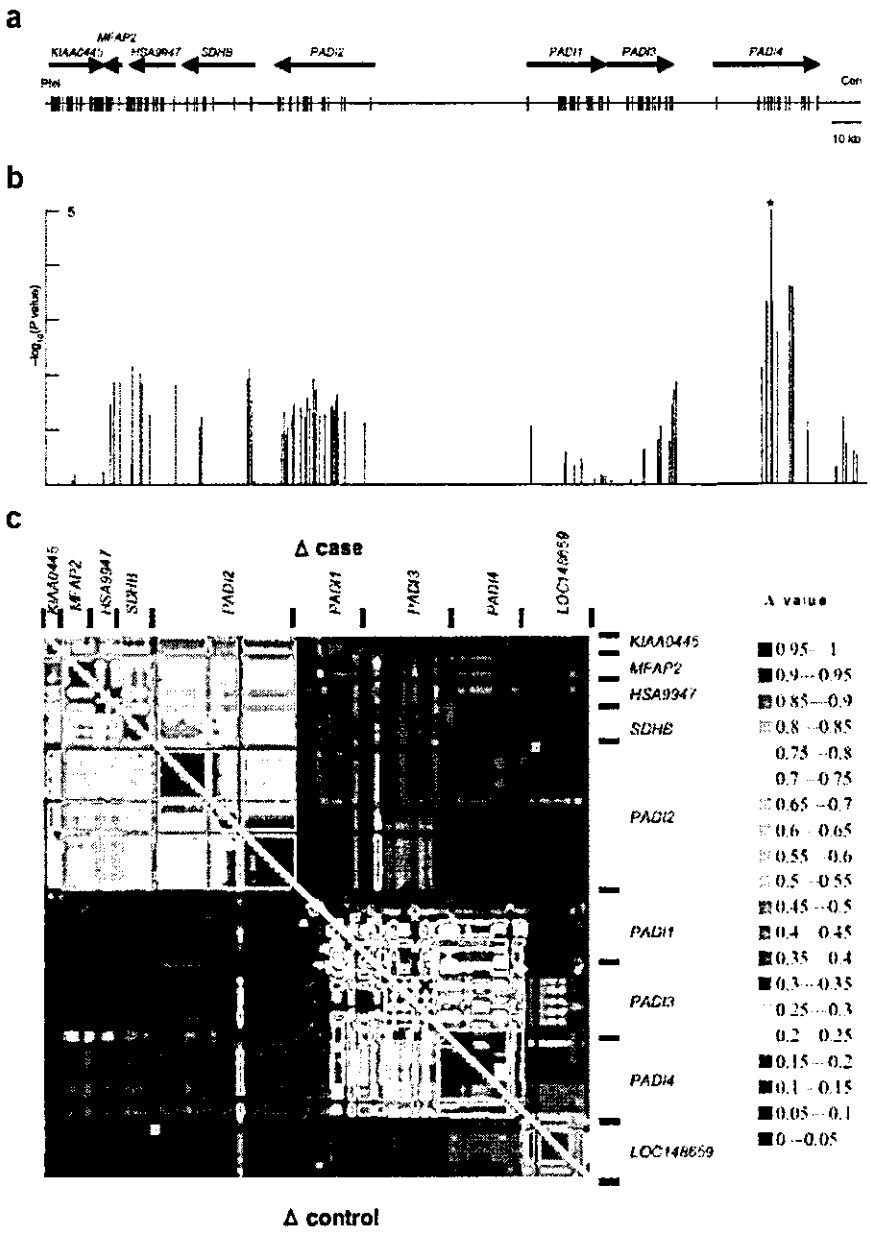

Figure 1 Gene content of NT_034367.1 in chromosome 1p36, case-control association and linkage disequilibrium. **(a)** Genomic structure of genes in this region. Ptel, p telomere; Cen, centromere. **(b)** Case-control association plots ($-\log_{10}$(*P* value)) versus location in this region. Asterisk indicates the SNP showing the strongest association in this region. **(c)** Pairwise linkage disequilibrium between SNPs, as measured by Δ in the case and control populations in this region: upper right triangle, case population; lower left triangle, control population.

RESULTS

Case-control study using SNPs in NT_034376.1

To identify genes associated with susceptibility to rheumatoid arthritis, we focused on the region NT_034376.1 on chromosome 1p36, in which we had previously identified the SNP strongly associated with rheumatoid arthritis. This region contains eight genes (including four *PADI* genes) that could be associated with rheumatoid arthritis according to the data regarding antibodies to citrullinated peptides. We refined the location of the rheumatoid arthritis susceptibility locus in a case-control study using 119 SNPs distributed in genes across contig NT_034376.1 (**Fig. 1a,b** and **Supplementary Table 1** online). The total length we evaluated was 445,670 bp, and SNPs were located every 3.7 kb on average. We predominantly used the Invader assay, which can efficiently detect genotypes of SNPs[17,18], and analyzed samples from a total of 830 affected individuals and 736 unaffected controls. Overall success rates of typing assays for cases and controls were 96% and 95%, respectively. A SNP in *PADI4*, padi4_94 (28017T in intron 3, susceptible; →C, non-susceptible), had the most significant association with rheumatoid arthritis (χ^2 = 19.856, P = 0.000008 comparing allele 1 versus allele 2; odds ratio (OR) = 1.97, 95% confidence interval (c.i.) = 1.44–2.69 comparing susceptible homozygotes versus non-susceptible homozygotes; **Table 1** and **Fig. 1b**). When Bonferroni's correction was applied to the result we obtained P = 0.00095, and the Monte Carlo Permutation test gave P = 0.00003 with 1×10^6 replications[19]. Both of these results were statistically significant.

We then sequenced all exons of *PADI4*, including the 5′ and 3′ untranslated regions, from 48 individuals with rheumatoid arthritis to identify SNPs. We identified four new SNPs and genotyped them in the exons: padi4_89 (163G→A in exon 2), padi4_90 (245T→C in exon 2), padi4_92 (335G→C in

Table 1 Summary of association between cases and controls in *PADI4*

	Genotype of case				Genotype of control				Allele 1 versus allele 2		Genotype 11 versus genotype 22
SNP ID	11	12	22	Sum	11	12	22	Sum	χ^2	*P* value	OR (95% c.i.)
padi4_92	166	416	241	823	102	307	246	655	12.36	0.00046	1.66 (1.23–2.25)
padi4_94	167	415	240	822	89	305	252	646	19.86	0.0000084	1.97 (1.44–2.69)
padi4_104[a]	268	355	110	733	313	358	64	735	12.67	0.00051	2.00 (1.41–2.86)[b]
padi4_95	131	386	304	821	64	300	281	645	12.29	0.00046	1.89 (1.35–2.66)
padi4_97	304	390	131	825	283	305	64	652	12.48	0.00041	1.92(1.35–2.70)[b]
padi4_99	225	421	181	827	224	331	100	655	13.72	0.00021	1.82 (1.33–2.44)[b]
padi4_100	225	418	180	823	216	332	98	646	12.00	0.00053	1.75 (1.30–2.38)[b]
padi4_101	222	417	178	817	216	322	95	633	13.62	0.00022	1.82 (1.33–2.50)[b]

Sum of cases > 800; $P < 0.001$.

[a]Control sample number of this SNP was 736. [b]For OR >1, the inverted score is indicated.

exon 3) and padi4_104 (349T→C in exon 4; **Table 1** and **Fig. 2a,b**). Overall, eight SNPs in NT_034376.1 had significant associations with rheumatoid arthritis ($P < 0.001$, **Table 1**), and all these SNPs were in *PADI4*. In the case and control populations, strong linkage disequilibrium extended only within *PADI4* and not to SNPs flanking *PADI4* (**Fig. 1c**). We therefore concluded that the strong association detected with SNPs in *PADI4* originated from *PADI4* itself. Rheumatoid factor status, sex, age at disease onset and *HLA-DRB1* status of affected individuals were not related to *PADI4* genotype distribution (data not shown).

We next undertook full haplotype analysis for 17 SNPs in *PADI4*. Only 4 of 2^{17} possible haplotypes were estimated to have frequency >0.02 in both case and control groups using the expectation-maximization algorithm. Less frequently occurring haplotypes were not shown, owing to concern over the accuracy of low frequency alleles in the expectation-maximization algorithm. The most frequently occurring haplotype, haplotype 1, and the second most frequently occurring haplotype, haplotype 2, comprised more than 85% of total chromosomes both in case and control groups (**Table 2**). Among the SNPs that segregate haplotype 1 and haplotype 2, four were exonic and three of them involved amino acid substitutions: padi4_89, padi4_90, padi4_92 and padi4_104, resulting in G55S, V82A, G112A and L117L, respectively (**Fig. 2c**). Haplotype 1 was more frequently observed in the control group and haplotype 2 in the case group. Haplotype 1 and its transcript and peptide were therefore termed 'non-susceptible', and haplotype 2 and its transcript and peptide 'susceptible'. Compositions of bases and amino acids of transcripts and peptides for susceptible and non-susceptible types are indicated in **Figure 2c**.

Expression of *PADI4* mRNA

To investigate the expression patterns of *PADI4* in tissues, we carried out northern-blot analysis and quantitative real-time RT–PCR. Northern-blot analysis identified two *PADI4* transcripts, one band at 2.6 kb and the other at 4.0 kb (**Fig. 3a**), as described in a previous study[20]. *PADI4* had high levels of expression in bone marrow and peripheral blood leukocytes, low levels of expression in spleen and fetal liver and no expression in other organs (including liver and kidney). *PADI4* was thus highly expressed in the organs of the hematological system.

We also confirmed *PADI4* expression in hematological cell types. Quantitative RT–PCR was done using RNA from $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, $CD14^+$ monocytes, polymorphonuclear leukocytes (PMNs), bone marrow and kidney (as a negative control). *PADI4* was highly expressed in bone marrow, $CD14^+$ monocytes and PMNs but was not expressed in $CD4^+$ and $CD8^+$ T cells or $CD19^+$ B cells (**Fig. 3b**).

Localization of *PADI4* mRNA, protein and citrullinated peptide

To test whether *PADI4* was expressed in rheumatoid arthritis synovial tissues, we carried out *in situ* RT–PCR. We observed *PADI4* mRNA in the lining or sublining layers of synovial tissues from all seven individuals with rheumatoid arthritis that we tested (**Fig. 3c**).

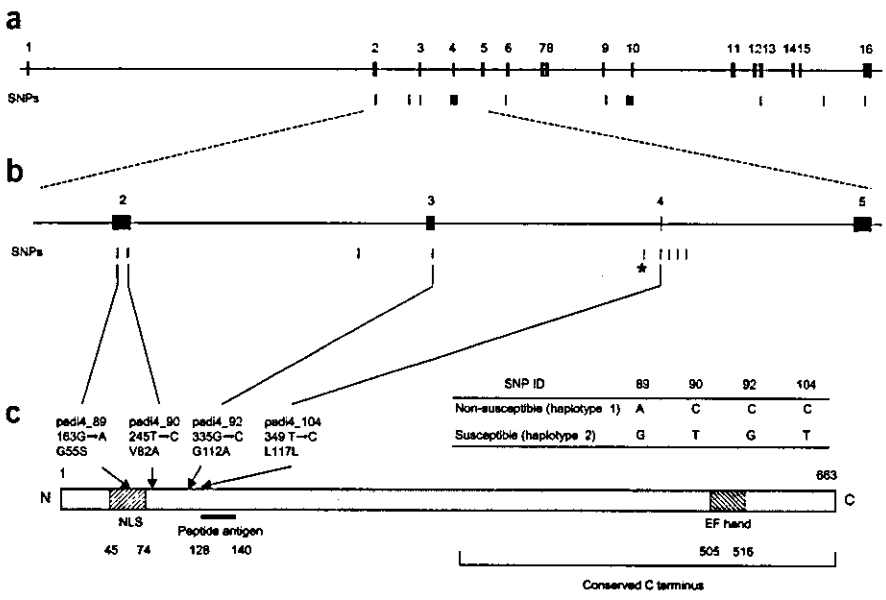

Figure 2 Structure of *PADI4*. (**a**) Exon-intron structure of *PADI4*. SNPs in *PADI4* are indicated below the gene. (**b**) Structure of region including exons 2–5. SNPs in this region are indicated below the gene. The asterisk marks the same SNP that is indicated in **Figure 1b**. (**c**) Protein structure of PADI4. Nucleotide numbering starts from start codons of genes. The bracketed region was used to generate the peptide antibody used in immunohistochemistry.

Table 2 Haplotype structure and frequency in *PADI4*

	Haplotype frequency		SNP ID (as padi4_x)															
Haplotype ID	Case	Control	89	90	91	92	93	94	104	95	96	97	98	99	100	101	102	103	105
Haplotype 1	0.52	0.60	A	C	C	C	C	C	C	G	T	T	C	A	T	T	C	T	C
Haplotype 2	0.32	0.25	G	T	T	G	A	T	T	C	C	A	T	G	C	C	C	C	C
Haplotype 3	0.06	0.04	G	T	T	G	A	T	T	C	C	A	T	G	C	C	T	C	C
Haplotype 4	0.06	0.04	G	T	T	G	C	T	C	G	T	T	C	G	C	C	C	T	C

We used sections of synovial tissues for immunohistochemistry with antibodies to PADI4 and to citrulline. In each sample from an individual with rheumatoid arthritis, PADI4 protein was detected in the sublining (**Fig. 3d**). Citrullinated peptide was also detected in the sublining with a similar pattern (**Fig. 3e**). These results indicate that PADI4 protein and citrullinated peptides are localized in rheumatoid arthritis synovia.

Stability of two types of *PADI4* mRNA

To investigate further the association between *PADI4* alleles and rheumatoid arthritis, we tested whether SNPs in exons affect the stability of *PADI4* mRNA. RNAs from the susceptible and non-susceptible alleles (**Fig. 2c**) were transcribed *in vitro* by modified RNase T1 selection assay[21]. Briefly, we mixed RNAs produced by *in vitro* transcription with extracts of HL-60 cells and observed the degradation of RNA by endogenous components of the HL-60 cell. Half-lives for susceptible and non-susceptible *PADI4* mRNA were 11.6 min and 2.1 min, respectively. Susceptible mRNA was therefore significantly more stable than non-susceptible mRNA (after 5 min, $P = 0.038$; after 10 min, $P = 0.017$; **Fig. 4**). Based on this result, mRNA stability seems to depend on haplotype.

Relationship between SNP and antibody to citrullinated filaggrin

Citrullination in proteins is believed to create epitopes recognized by rheumatoid arthritis autoantibodies that not only represent the most specific serologic markers, but also appear early[22], even before clinical onset of rheumatoid arthritis. Citrullinated filaggrin has been used in clinical laboratory tests as a possible candidate for citrullinated autoantigens[23]. We therefore examined the relationship between *PADI4* haplotype and the presence of antibodies to citrullinated filaggrin in sera from individuals with rheumatoid arthritis. Individuals homozygous with respect to the susceptible haplotype were more likely to be positive (87%) for antibody to citrullinated filaggrin than the other two genotypes, for whom the positive fraction rate was 50% (**Table 3**). This tendency was tested using Fisher's exact test and was marginally significant (**Table 4**, $P = 0.038$).

DISCUSSION

A genome-wide association study to identify genes associated with rheumatoid arthritis is in progress in Japan using a high-throughput multiplex PCR-Invader assay[17,18]. Although the project has not yet been completed, one candidate locus has been identified in contig NT_034376.1. Previous sibling-pair linkage studies have also shown that this region is one of the three strongest susceptibility loci for rheumatoid arthritis[5,8]. This locus contains all four identified *PADI* genes, which encode calcium-dependent enzymes that catalyze the conversion of arginine to citrulline in peptides. This activity itself suggested that *PADI* genes may be involved in rheumatoid arthritis, and the antibodies are the most specific rheumatoid arthritis–specific antibodies identified[23–26]. Although several other genes with functional association to rheumatoid arthritis, including that encoding tumor necrosis factor receptor 2 (ref. 27), have been localized to 1p36, *PADI* genes were considered the most relevant for investigation owing to the rheumatoid arthritis specificity of the autoimmune response to citrullinated epitopes.

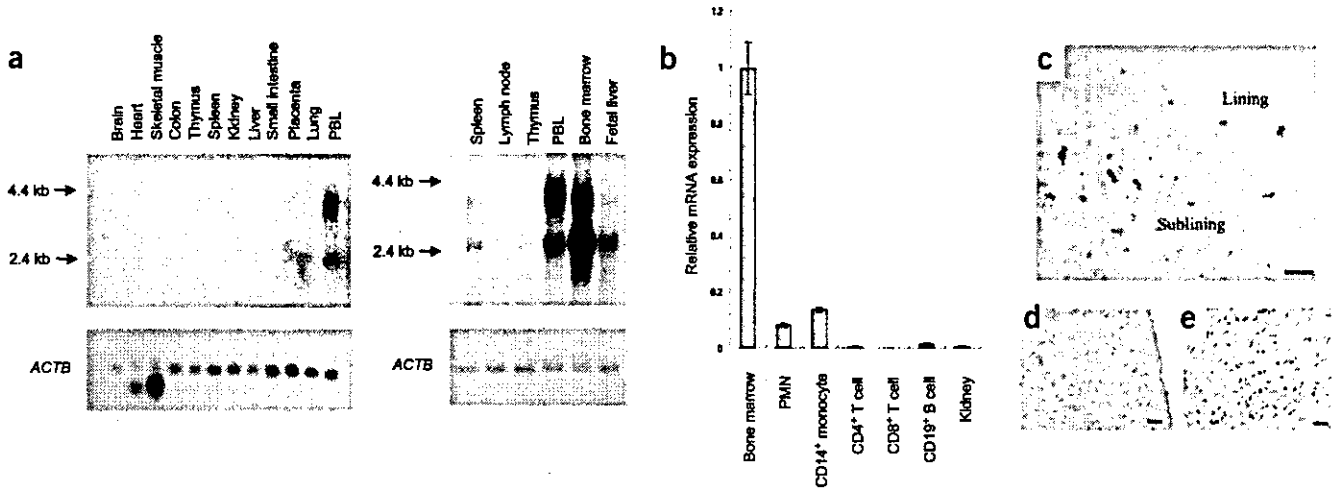

Figure 3 Expression of *PADI4*. (**a**) Expression of *PADI4* mRNA in various normal human tissues. (**b**) Relative expression level of *PADI4* mRNA in normal human tissues and cells. Values represent mean ± s.d. of data from triplicate wells. (**c**) Expression and distribution of *PADI4* mRNA in rheumatoid arthritis synovial tissue as analyzed by *in situ* RT–PCR. *PADI4* transcript (dark blue) was stained in sublining and lining. Immunohistochemistry showing expression patterns of PADI4 (**d**, red stain) and citrullinated peptides (**e**, red stain) in rheumatoid arthritis synovium. No non-rheumatoid arthritis tissue control was used. Scale bars: **c**, 250 μm; **d**, **e**, 100 μm.

We identified eight genes in contig NT_034376.1, including four *PADI* genes (**Fig. 1a**). We evaluated the strength of association with rheumatoid arthritis across the region by linkage disequilibrium mapping of 119 SNPs (**Fig. 1b**). The association in the region was definitive ($P = 0.000008$, OR = 1.97, 95% c.i. = 1.44–2.69) and was considered to originate in *PADI4*, rather than any other *PADI* gene (**Fig. 1c**). We observed a similar pattern of linkage disequilibrium in cases and controls, which is consistent with the association pattern (**Fig. 1c**) and provides additional support for *PADI4* as the origin.

An OR of 1.97 suggests that the genetic contribution of *PADI4* is not as strong as that of the *HLA-DRB* locus (OR = 2.60, 95% c.i. = 1.88–3.60; ref. 28) but is nonetheless considerable. The *HLA-DRB* locus has been estimated to explain less than or close to half of the total genetic contribution to rheumatoid arthritis, with the remainder attributed to multiple non-*HLA* genes[1]. We therefore expect that *PADI4* is one of the primary non-*HLA* genes associated with rheumatoid arthritis. A genotypic risk ratio for *PADI4* is 1.3 (ref. 29), and its population attributable risk is 17.4% (ref. 30), which seems reasonable for a gene associated with a complex genetic trait like rheumatoid arthritis. Furthermore, a locus with this degree of genetic contribution could be detectable in linkage studies, as was the case for microsatellite markers close to *PADI4* in two linkage studies[5,8].

Northern-blot analysis indicated that *PADI4* was highly expressed in bone marrow and peripheral blood leukocytes. Quantitative RT–PCR indicated that *PADI4* mRNA is expressed in PMNs, which include neutrophils and the monocyte lineage, but is not expressed in lymphocytes. Previous reports have shown high *PADI4* expression in neutrophils, eosinophils and monocytes[20,31]. *PADI4* is therefore expressed in hematological tissues and cell types, which are known to be intimately involved in the pathogenesis of rheumatoid arthritis[32,33]. Although the importance of antigen-specific immune processes has been emphasized in the investigation of rheumatoid arthritis, the finding that myeloid leukocytes, rather than lymphocytes, are the predominant cell types in which *PADI4* is expressed indicates that more investigation of the roles of myeloid lineages in rheumatoid arthritis is warranted.

We examined expression of *PADI4* in synovial tissues of seven individuals with rheumatoid arthritis using *in situ* RT–PCR and immunohistochemistry. Both mRNA and protein were expressed in the sublining region, and both PADI4 protein and citrullinated peptide were localized in the sublining region. A previous study reported citrullinated α- and β-fibrin in sublining regions of fibroblast- and macrophage-like mononuclear cells of individuals with rheumatoid arthritis[14]. Peptides in synovial tissues, including fibrins, were proposed to be citrullinated by PADI4 extra- or intracellularly with subsequent secretion, behaving as autoantigens recognized by rheumatoid arthritis–specific antibodies. Lining regions contained *PADI4* mRNA but no protein. The reason for this discrepancy is unclear. Collectively, these data suggest that citrullination by PADI4 occurs in the sublining of synovial tissues and that citrullinated peptides behave as antigens for rheumatoid arthritis–specific autoantibodies. Although the detection of *PADI4* expression in rheumatoid arthritis synovial tissue without comparison to non-rheumatoid arthritis controls does not imply that expression and activity of *PADI4* are specific to rheumatoid arthritis, its presence does support other findings that link rheumatoid arthritis and *PADI4*.

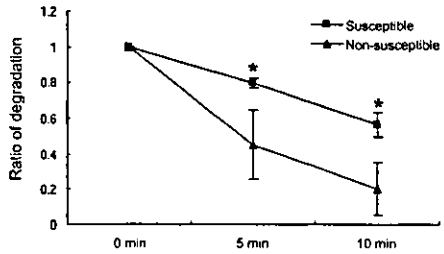

Figure 4 Stability of susceptible and non-susceptible transcripts of *PADI4* mRNA measured as degradation rate. Differences were significant (*$P <$ 0.05) after 5 min and 10 min of reaction time. Values represent mean ± s.d. of data from duplicate experiments.

To investigate the relationship between pathogenesis of rheumatoid arthritis and haplotypes comprising the four SNPs in *PADI4* mRNA (**Fig. 2c**), we examined whether these SNPs affect *PADI4* mRNA stability. The mRNA of the susceptible haplotype was more stable than that of the non-susceptible haplotype (**Fig. 4**). In previous studies, SNPs in mRNA or one-base deletions in coding regions have been associated with transcript stability[34,35]. The present result also suggests that SNPs in mRNA contribute to mRNA stability. Susceptible-haplotype mRNA probably accumulates to higher levels than non-susceptible mRNA, resulting in higher levels of PADI4 protein. Stable *PADI4* mRNA may increase PADI4 proteins in synovial tissues, neutrophils and monocytes, increasing production of the citrullinated peptides that serve as autoantigens. Apart from stability of transcripts, evaluation of the effects of substitution of amino acids on the enzyme is important and further investigation should be directed at such analyses. Although SNPs in exons were systematically searched and the effect of coding SNPs analyzed in this report, involvement of other polymorphisms in non-coding regions is possible[35]. Further investigation in intron regions and other regulatory areas is therefore desirable.

The relationship between *PADI4* and rheumatoid arthritis is further supported by the fact that the positive fraction of antibodies to citrullinated peptides was significantly higher in individuals homozygous with respect to the susceptible haplotype than in those of other genotypes ($P = 0.038$, **Table 4**). The present study yielded statistically significant results only in comparing susceptible homozygotes with others and not in comparing non-susceptible homozygotes with others. The absence of a significant difference in the latter comparison might be due to the small number of samples or the mixture of individuals with positive results irrelevant to rheumatoid arthritis–related PADI activity, as should be observed in healthy controls using a test with sensitivity of 75.6%. Previous reports that antibodies to citrullinated peptide are specific to rheumatoid arthritis and are detectable in the early phases of the disease[36] suggest that citrullination by

Table 3 Distribution of individuals of each genotype that were positive for antibody to citrullinated filaggrin

SNP genotype			
Antibody to citrullinated filaggrin	Susceptible homozygotes	Heterozygotes	Non-susceptible homozygotes
Positive	26 (30%)	40 (45%)	22 (25%)
Negative	4 (11%)	20 (57%)	11 (11%)
Positive fraction	0.87	0.50	0.50

Table 4 Association test between genotype and antibody positivity

Comparison pattern	*P* value*
Susceptible homozygotes versus others	0.038
Non-susceptible homozygotes versus others	0.468

**P* value was calculated by Fisher's exact test (two-tailed).

PADI4 should be closely linked to onset of rheumatoid arthritis or might represent a disease-triggering event in itself.

To investigate the precise role of *PADI4* in rheumatoid arthritis, we evaluated the mouse homolog of *PADI4*, *Padi4*, in a collagen-induced arthritis (CIA) mouse model. Expression of *Padi4* was quantified (**Supplementary Fig. 1** online). We induced expression of *Padi4* in inflamed synovial tissues and spleen in mice with CIA. In humans, genotype with respect to *PADI4* was associated with rheumatoid arthritis, presence of PADI4 in affected joints was detected and antibody to citrullinated peptide was detected in sera. In mice, expression of *Padi4* increased with appearance of CIA, but antibody to citrullinated peptide was not detected in sera (data not shown). The primary difference between human rheumatoid arthritis and mouse CIA is that the former is characterized by breakdown of self-tolerance and continuity of destructive arthritis with accompanying autoimmune phenomena to various autoantigens including antibody to citrullinated proteins, whereas the latter shares the inflammatory component related to immune response to collagen type II with rheumatoid arthritis, but specificity of its immunoreaction is higher and breakage of tolerance to citrullinated antigens does not seem to be involved. Given the results of the present study, we consider citrullination by PADI4 or Padi4 as one of the processes in early phase arthritis, and that, in human rheumatoid arthritis, immunological tolerance breaks down somehow with the appearance of autoantibody recognizing citrullinated peptide, followed by the autoimmune disease process characterized for rheumatoid arthritis. In mouse CIA, however, expression of *Padi4* increases with a probable increase in citrullination of self-peptides, but tolerance to citrullinated-antigens does not seem to break. Even with these differences in mechanisms between human rheumatoid arthritis and mouse CIA, further investigation of *PADI4* in human rheumatoid arthritis and *Padi4* in the mouse model seems warranted.

In conclusion, we identified *PADI4* as a susceptibility gene for rheumatoid arthritis using a case-control study with SNPs. The present findings imply that the rheumatoid arthritis susceptibility haplotype in *PADI4* produces a more stable transcript and is associated with higher levels of antibody to citrullinated peptide in sera of individuals with rheumatoid arthritis. Given the polygenic nature of rheumatoid arthritis, this independent susceptibility gene could have a most important role in rheumatoid arthritis pathogenesis by increasing citrullination of proteins in rheumatoid arthritis synovial tissues, leading, in a cytokine-rich milieu, to a break in tolerance to citrullinated peptides processed and presented in the appropriate *HLA* context.

METHODS

Subjects with rheumatoid arthritis and unaffected subjects. We recruited a total of 830 individuals affected with rheumatoid arthritis and 736 unaffected controls for collection of genomic DNA and sera through several medical institutes in Japan. We sampled pathological joint synovial tissues from seven individuals with rheumatoid arthritis who underwent arthroplasty surgery. All rheumatoid arthritis cases met the revised criteria of the American College of Rheumatology for rheumatoid arthritis[37]. The mean age of the 830 case individuals with rheumatoid arthritis was 64.3 y (range, 28–92 y). Most case subjects were female (83.7%), and 75% were positive for rheumatoid factor. Control subjects comprised 736 individuals from the general population, 57.4% females, with mean age of 48.6 y (range, 3–92 y). We obtained informed consent from each subject, with parental authority in the case of minors, as approved by the ethical committee of the SNP Research Center of The Institute of Physical and Chemical Research (RIKEN).

SNPs. We identified four SNPs in exons of *PADI4* and 14 SNPs in *LOC148695* by direct sequencing of DNA from 48 case individuals. We selected the other 101 SNPs, which were located in genes (promoter, exon and intron) in NT_034376.1 (gi: 22043311) from the JST database.

Genotyping. We extracted genomic DNA from peripheral blood leukocytes using standard protocols[17]. We genotyped SNPs using the Invader assay, TaqMan assay or direct sequencing. For Invader assay, we amplified DNA with PCR primers designed to include one or more SNPs, as previously described[18,38]. Third Wave Technologies designed probe sets for each locus. In TaqMan assay, we carried out PCR using TaqMan Universal Master Mix (Applied Biosystems), 8 ng DNA, 1 μM of each primer and 200 nM of probe in 15-μl reactions. Each 96-well plate contained 94 samples of unknown genotype and 2 no-DNA control samples. Thermal cycle conditions were 50 °C for 2 min, 95 °C for 10 min, 50 cycles of 92 °C for 15 s and 58 °C for 1 min. Thermal cycling was done on an ABI PRISM 7700 Sequence Detector Systems (Applied Biosystems). We undertook direct sequencing of PCR products using ABI3700 capillary sequencers (Applied Biosystems) according to standard procedures.

Northern-blot hybridization. We hybridized human multiple tissue northern (MTN) blots (Clontech) with a *PADI4* probe labeled with digoxigenin. We generated digoxigenin-labeled *PADI4* probes using a PCR digoxigenin probe synthesis kit (Roche Diagnostics) according to the manufacturer's instructions, using the primers to generate a 335-bp product. Hybridization and detection were also done according to the manufacturer's instructions. Blots were stripped of probe and re-hybridized with a cDNA probe for *ACTB* (Roche Diagnostics) to assess RNA loading. Primer sequences are available on request.

RNA extraction and cDNA synthesis. We separated PMNs using Mono-Poly resolving solution (Dainippon Pharmaceuticals) and extracted RNA from PMNs using ISOGEN (Nippon Gene). We stored the resulting RNA at −80 °C until use. We quantified RNAs of other normal tissues using Premium total RNA (Clontech). We reverse-transcribed total RNA (1 μg) using a First Strand cDNA synthesis kit (Amersham Pharmacia) according to the manufacturer's instructions.

Quantification of *PADI4* expression by real-time RT–PCR. We carried out real-time PCR on the ABI PRISM 7000 (Applied Biosystems) using QuantiTect SYBR Green PCR (QIAGEN) according to the manufacturer's instructions. Each oligonucleotide primer set was added to a final concentration of 0.3–0.5 μM for *ACTB* (product size, 219 bp) and *PADI4* (product size, 207 bp). We generated a standard curve from data of amplification of *PADI4* primers using a dilution series of bone marrow mRNA as templates and normalized to *ACTB*. Primer sequences are available on request.

***In situ* RT–PCR.** We carried out one-step *in situ* RT–PCR by adding Pro STAR HF (Stratagene), and reactions were done using an Omnislide thermal cycler (Hybaid) as follows: (i) 42 °C for 30 min; (ii) 94 °C for 2 min, 55 °C for 45 s and 68 °C for 2 min; and (iii) 25 cycles at 94 °C for 45 s, 55 °C for 45 s and 68 °C for 2 min. Reactions were maintained at 4 °C after amplification. After PCR, we washed slides twice with Tris-buffered saline for 5 min. Specific primers amplified their specific target sequences, yielding a 335-bp product.

We generated digoxigenin-labeled internal probes by PCR using the PCR digoxigenin probe synthesis kit according to the manufacturer's specification with minor modifications. We added primers to a final concentration of 0.34 μM. We covered slides with pre-hybridization solution at 37 °C for 1 h. After pre-hybridization, we replaced pre-hybridization solution with hybridization

solution containing probe. Probes were denatured at 94 °C for 5 min. We carried out hybridization for 12 h at 37 °C. After washing, we visualized incorporated PCR fragments using a digoxigenin detection kit (Roche Diagnostics).

Controls included several different samples, substituting water for primer in the PCR reaction, omitting reverse transcription in the case of mRNA and omitting probe in hybridization solutions (X.C. *et al.*, manuscript in preparation). Primer sequences are available on request.

Preparation and purification of antiserum against PADI4. We synthesized *PADI4*-derived peptides (Sp-PAD1: PAKKK STGSS TWP-Cys), purified and immunized in rabbits (Kitayama-Labes, Nagano, Japan). We purified antiserum by affinity chromatography on a histidine-tagged PADI4 column (BioGate). We confirmed specificity of purified polyclonal antibody to PADI4 with western blotting using a transient expression system in the HEK293 cell line (data not shown).

Immunohistochemistry. We incubated paraffin sections of synovial tissues at 4 °C for 12 h with rabbit polyclonal antibody to PADI4 or with rabbit antibody to citrulline (Biogenesis), diluted at 1:1,000. We washed and incubated sections at room temperature with Simple Stain MAX-PO (Nichirei) for 30 min and then added Simple Stain AEC (Nichirei). We incubated sections for 5–20 min until the reaction was obviously visible under light microscopy. All sections were counterstained with hematoxylin. In all cases, negative controls omitted the specific antibody and used normal mouse and rabbit antiserum.

Measurement of antibody to citrullinated filaggrin. We measured levels of antibody to citrullinated filaggrin using an ELISA kit (MBL) according to the manufacturer's instructions. Sensitivity was 75.6% and specificity was 83.2% for testing subjects with and without rheumatoid arthritis n clinical settings at a cutoff level of 9 (K. Suzuki *et al.*, manuscript accepted).

***In vitro* RNA stability assay.** We amplified genes encoding two *PADI4* variants by PCR from cDNAs that were synthesized using a first-strand cDNA synthesis kit (Amersham Pharmacia) with bone marrow total RNA (Clontech). We then cloned these genes into the pDONR201 vector (Invitrogen). We also constructed the cDNA into pDEST14 (Invitrogen), which has a T7 promoter, and sequenced both strands of the resulting expression vector. Vectors were digested using *Cla*I, and both types of *PADI4* were expressed using RiboMax Large Scale RNA Production System-T7 (Promega) and purified according to the manufacturer's instructions. To prepare whole-cell extract, we washed HL-60 cells in phosphate-buffered saline and re-suspended them in extraction buffer (0.5% Nonidet P-40; 20 mM HEPES buffer, pH 8.0; 20% glycerol (v/v); 400 mM NaCl, 0.5 mM dithiothreitol; 0.2 mM EDTA and 1% protease inhibitor cocktail (Nacalai)). After incubation on ice for 30 min and microcentrifugation at 4 °C, we transferred supernatants to new tubes and stored them at –80 °C until use.

We mixed and incubated each 5 μg of synthesized RNA and diluted whole-cell extract (1:1,000) at room temperature. The reaction was stopped with the addition of formamide dye, and the samples were then heated at 68 °C. After the reaction, we detected RNA using northern-blot hybridization. We scanned results on a DocuCentre Color 500cp (Fuji-Xerox) and measured signal intensities of full-length RNAs using Adobe Photoshop 6.0.

Statistical analysis. We estimated haplotype frequencies using the expectation-maximization algorithm[39]. We calculated linkage disequilibrium index, Δ (ref. 40), and drew **Figure 1c** with an application created by our group with the assistance of Excel (Microsoft). Associations between phenotypes were estimated by χ^2 test. Antibody to citrullinated filaggrin titer and genotypes were tested using Fisher's exact test on Statistica software (StatSoft), and mRNA stability data and quantitative RT–PCR data were tested using Student's *t*-test.

URLs. The National Center for Biotechnology Information can be found at http://www.ncbi.nlm.nih.gov/. The International RH Mapping Consortium can be found at http://www.ncbi.nlm.nih.gov/genemap99/. The expectation-maximization program can be found at http://linkage.rockefeller.edu/ott/eh.htm.

GenBank accession numbers. *PADI4*, NM_012387; *LOC148695*, XM_088976; *Padi4*, NM_011061.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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関節リウマチ頚椎手術に関する全国調査

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【目的】近年の麻酔・全身管理の進歩，脊椎手術手技・インストゥルメンテーションの発展は，関節リウマチ(RA)頚椎病変に対する手術治療の安全性や有効性にも寄与し，良好な成績報告が増加している．しかしRAの日常診療は主に内科医または関節外科医が担当しており，頚椎病変による臨床症状が重度に進行してから紹介され，手術治療の効果が十分に享受できない症例も散見される．これまでRA頚椎手術の全国的な調査報告はなく，平成14年に発足した「RAの頚椎・上肢機能再建に関する研究班」では，まず疫学調査の必要性を認識した．本研究の目的は，本邦のRA頚椎手術数，手術方法，脊椎手術への経緯等を明らかにすることである．【方法】RA頚椎手術と最も関連がある日本脊椎脊髄病学会に依頼し，同学会の脊椎手術に関する障害予防委員会が行う全国レベルの脊椎手術合併症調査(平成14年12月に主な施設へ依頼)にあわせて，本調査を実施した．調査対象は平成13年施行のRA頚椎手術症例で，調査内容は，年齢，性，手術方法，神経症状(Ranawatのclass)，RA薬物治療実施医，脊椎外科医への紹介経緯で，合併症調査と同一のファイルメーカー内に入力し回答を得る形式とした．調査の配信，回収は障害予防委員会が行い，依頼施設数は313，回答施設数は196（回答率62.1%）であった．【結果】回答を得たRA頚椎手術は234名236手術であった．初回手術例218名(2名の予定二期手術例を含む)，再手術例16名であった．性別は男性41名，女性190名，不明3名で，年齢は33～85歳(平均63.4歳)であった．実施施設数は74施設で，年間3例以下が55施設と大半を占め，10例以上は1施設のみであった．手術法を3群に分類した．後頭骨からの固定術(O-C固定)96手術，環軸椎固定術(C1-2固定)102手術，その他の手術38手術である．O-C固定群の下位固定椎はC1からT5に及んでいたが，主にC2～3(34例)とC7～T2(36例)の2部位に分かれた．C1-2固定群には，その単独例89例と中下位頚椎除圧術を併用した13例を含めた．なお環軸椎固定方法はMagerl法が66例と最も多く，wiringのみは30例であった．その他の手術は，椎弓切除・形成の単独または固定の併用が28例で，前方固定単独6例，経口除圧術は1例のみであった．Ranawatの神経症状classは，I：30名，II：43名，IIIa：55名，IIIb：30名，不明：29名，回答なし：47名であった．なお不明，回答なしを除くと，O-C固定群はIIIa，IIIbが73.5%を占め，C1-2固定群はI，IIが69.8%を占めた．RA薬物治療実施医は，整形外科医111例，内科医71例，その他4例，なし1例，回答なし47例であった．手術経緯は，同一施設での治療例74例，他施設からの紹介例104例，その他13例，回答なし43例であった．リウマチ科・内科からの経由は55例，整形外科経由は117例であった．【考察】RA頚椎手術は脊椎手術の中でも専門化された分野であり，また脳神経外科医の脊椎手術への参入が活発になってきているとはいえ関節外科と関連の深い本疾患は整形外科脊椎専門医の手術関与の可能性が圧倒的に高いことを考慮すると，今回の調査依頼は主に脊椎手術を相当数実施している施設の整形外科に実施しているが，RA頚椎手術の大半の症例を抽出していると考える．年間手術数は236であったが，単独施設での症例数は限られており，頚椎病変の多様性も考慮すると，今後その治療成績を科学的根拠に基づき議論していくには多施設共同での症例蓄積が必須であると結論できる．また選択された手術法により術前の神経症状重症度に違いがみられ，これは疾患の多様性と現状での各手術の選択の問題を反映していると考えられ，手術を画一的に評価することができないことを示唆させる．今回の結果の比較対象となるデータは少ないが，手術例のRA薬物治療実施医が整形外科医47.4%，内科医30.3%という結果は，リウマチ友の会による2000年リウマチ白書での主治医が整形外科系49.8%，内科系33.1%という結果とほぼ同等であった．これはRA頚椎病変に対する内科系医師の認識の高まりを反映している可能性を推測させるが，引き続きRA治療医に対する頚椎病変の重要性の啓蒙は必要であると考える．本調査は，平成14年に発足した厚生労働科学研究：免疫アレルギー疾患予防・治療研究事業「関節リウマチの頚椎・上肢機能再建に関する研究」班が実施した．本調査にあたりご協力頂いた日本脊椎脊髄病学会脊椎手術に関する障害予防委員会，回答を頂いた全国の諸施設に対し深謝致します．

An epidemiologic survey about cervical spine surgery in rheumatoid arthritis

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Key words : rheumatoid arthritis, cervical spine surgery, epidemiology