を作成することに成功した．このような手法を用いれば，後発白内障を透明化したり，水晶体を再生させることが可能になると期待される．

おわりに

分子生物学の進歩により，水晶体形成に関わる遺伝子群と疾患におけるそれらの変異が明らかになってきている．水晶体の形成を理解するうえでも，将来の再生医学応用に向けても，水晶体形成遺伝子カスケードとこれらを誘導するシグナル伝達物質など細胞環境の詳細を明らかにすることが現在の課題である．

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未熟児網膜症

東　範行　国立成育医療センター眼科

要旨

未熟児網膜症は，低出生体重児の管理の進歩に伴って，近年増加しており，重症例も多くみられるようになった．定期的な眼底検査を行い，病期分類をよく理解して，重症例を見逃がさないことが重要である．治療は中等度網膜症であれば光凝固を，網膜剥離が進行すればバックリングや硝子体手術を行う．

Key Words

未熟児網膜症
病期分類
光凝固
硝子体手術

はじめに

未熟児網膜症は発達途上の網膜血管が増殖する疾患で，重症であれば失明に通ずる．網膜血管は胎齢15週に視神経乳頭部に現れ，眼底を周辺部にむかって成長していく．血管が眼底の最周辺部まで達するのは満期の40週頃なので，発育途上で出生して急な環境変化があると，網膜血管は異常な方向に増殖する．したがって，網膜症の発現頻度や程度は血管成長が未熟であるほど高いが，ほかにも発病に関する多くの因子がある．未熟児網膜症は，NICUでの管理の進歩によって一時減少していたが，体重の少ない児が救えるようになって[1)2)]再度増加し，重症網膜症も多くみられるようになった[3)-5)]．

未熟児網膜症の進行と病期分類

未熟児網膜症の初期は，血管成長先端部の網膜内で血管芽細胞が増殖を始め，白い境界線を形成する（図1）．やがて境界線上やその後部で新生血管が発芽し，しだいに融合して硝子体腔内へ伸びていく（図2）．

眼底では乳頭は鼻側に位置しており，網膜血管が乳頭から周辺まで成長する距離は鼻側に比べて耳側が長いので，耳側のほうで網膜症がおこりやすい．さらに進行すると，網膜剥離がお

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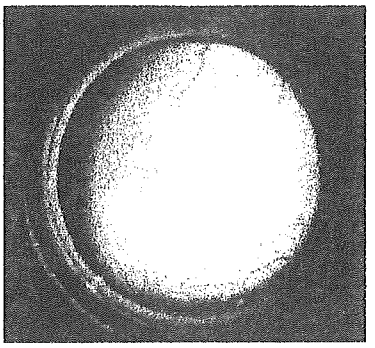

図1　境界線（眼底写真）

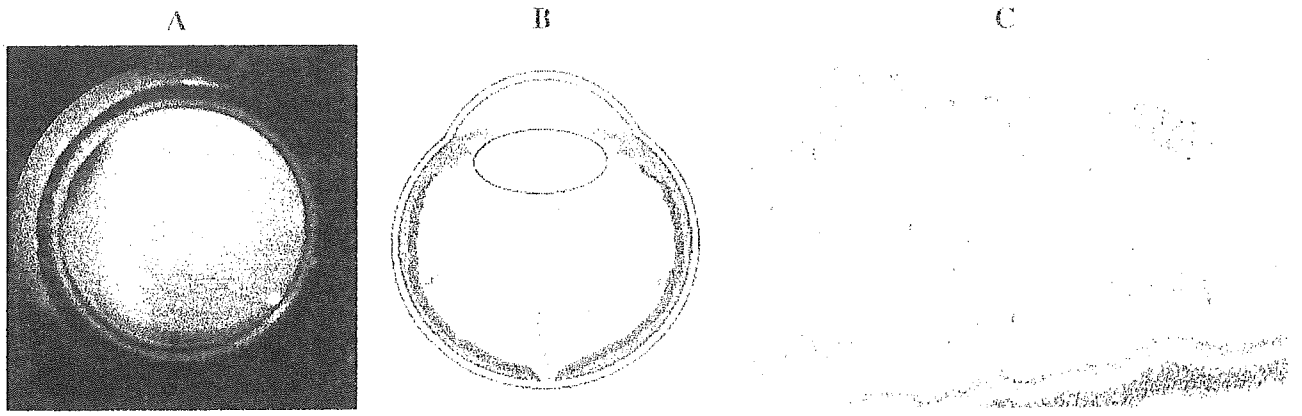

図2　発芽病変
A：眼底写真，B：眼球シェーマ，C：病理所見

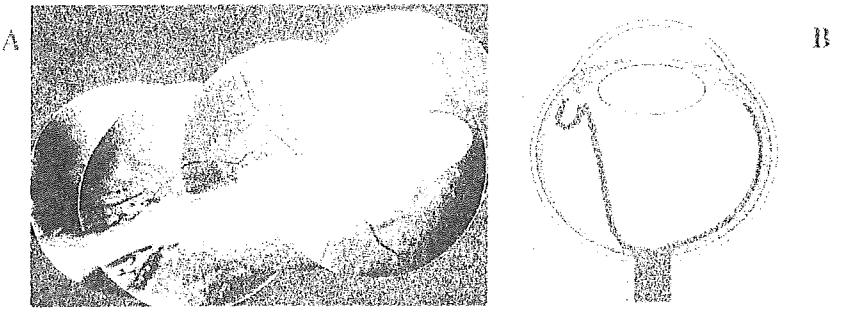

図3　網膜ひだ
A：眼底写真，B：眼球シェーマ

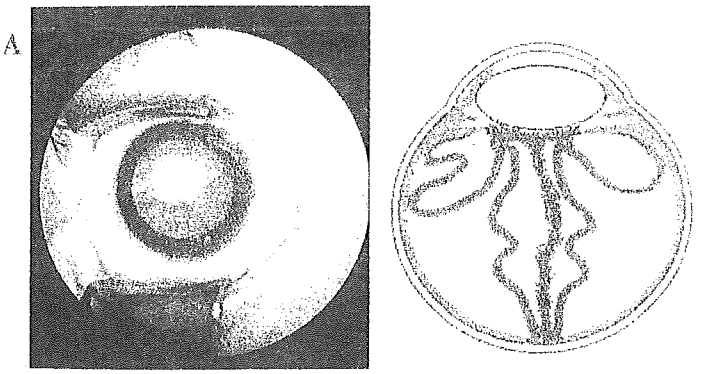

図4　白色瞳孔を示す高度な増殖による網膜全剝離
A：眼球前方の写真，B：眼球シェーマ

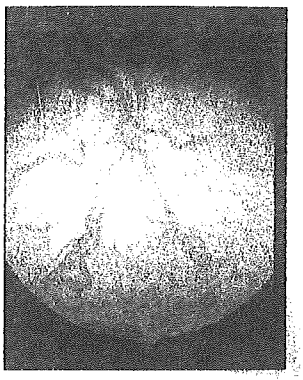

図5　Ⅱ型網膜症の後極血管の拡張と蛇行

こる．これは新生血管から形成された結合組織の収縮による牽引性剝離と，血管からの漏出による滲出性剝離の2種類がある．増殖組織が一側に限局していれば，網膜はそちらに引かれて伸展し，牽引乳頭や網膜ひだ（図3）を形成する．

高度な増殖がおこれば網膜は全剝離し，白色瞳孔を呈するようになる（図4）．ことに，網膜血管の成長が不良で拡張蛇行が強い場合は，短期間に進行して網膜全剝離になるおそれがある（厚生省分類Ⅱ型，図5）

この進行病期に関して，わが国では1976年に厚生省研究班によって『未熟児網膜症の診断ならびに治療基準』[6]が作成され，1983年には一

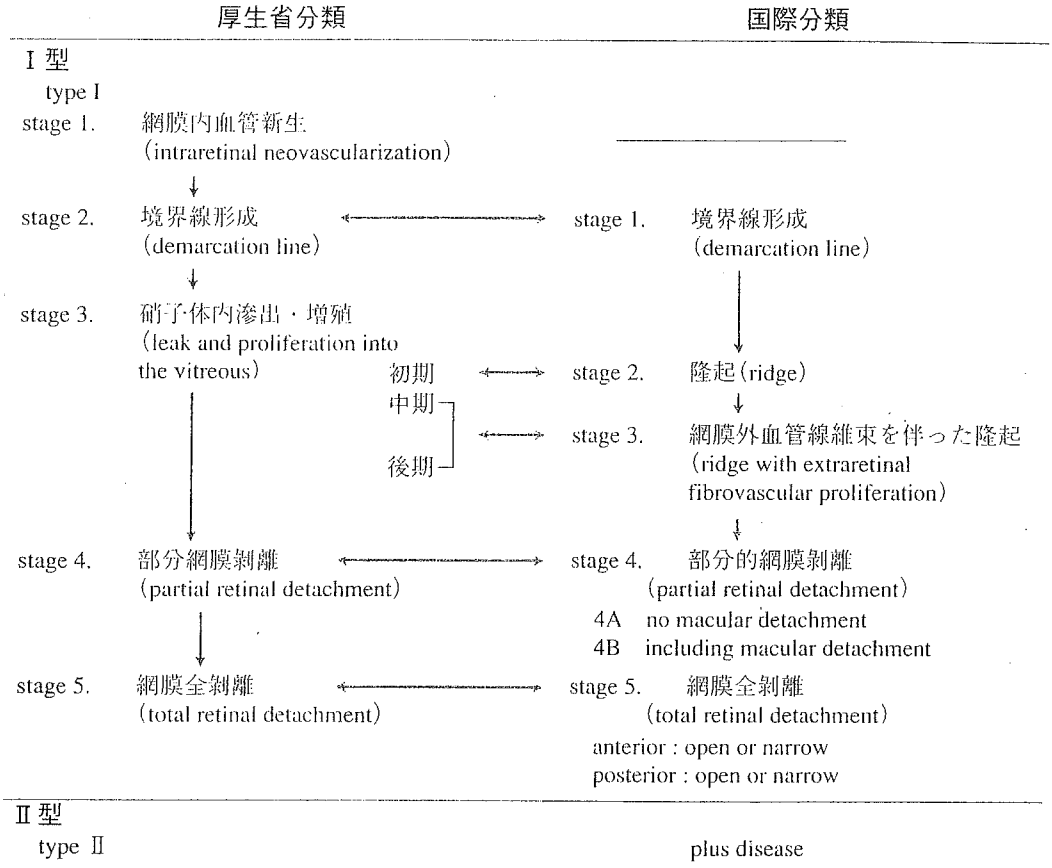

図6　厚生省分類と国際分類

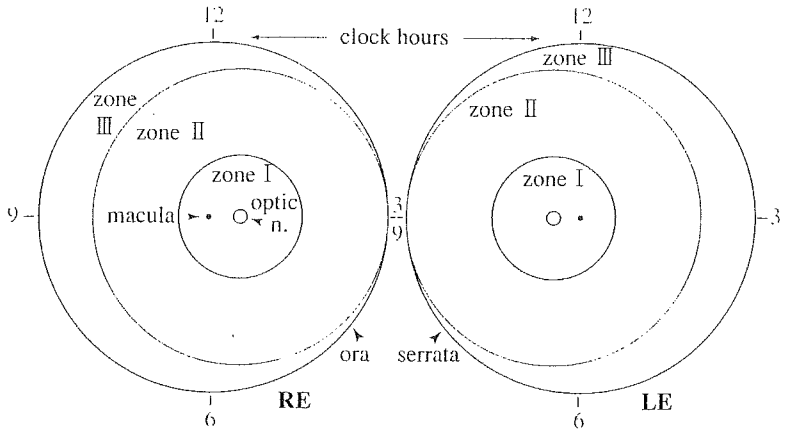

図7　国際分類の眼底 zone

部修正して，『未熟児網膜症厚生省新分類』[7]となり，現在広く使われている．これと前後して，わが国を含む未熟児網膜症の研究者が集まって国際分類作成を行い，1984年[8]と1987年[9]に発表された．厚生省分類と国際分類はstage 1とstage 2の扱いが異なる．わが国では厚生省分類が広く定着しているが，国際分類への書き換えは容易である．両分類の比較を図6に示す．

国際分類では検査結果をコンピュータに入力可能にできるようにするため，stageを5期に分け，眼底を三つのzoneに分けて病変の局在と範囲を記載するようにした（図7）．一方，厚生省分類では急速に進行して網膜剥離に至る重症網膜症をⅡ型としているが，国際分類ではこの概念がない．後極部静脈の怒張，動脈の蛇行，虹彩血管の充血や瞳孔強直が高度な場合は網膜症の進行が早く重篤なので，これを“plus” diseaseとして，『＋』の記号をつけるようにしているが，厚生省分類Ⅱ型とは異なる．最近，欧米でもこのⅡ型が認識されるようになってきた．さらに，わが国では網膜症が寛解し瘢痕を残した場合の瘢痕期分類が作成されているが，国際分類では記載する瘢痕病変の項目のみにとどめている．

未熟児網膜症の発生に関与する因子

網膜血管は周産期に眼底周辺部に達するが，未熟な血管形成部は，数カ月にわたって原始的な毛細血管網から成人の形態に作り変えられ，通常生後2～3カ月に完成する．未熟児で出生した場合，出生と以後の環境の変化に伴うストレスによって，網膜内の発達過程にある毛細血管床が傷害され消失し，そこから新生血管がおこる．したがって，網膜症の発生にもっとも大きく関与する因子は網膜血管の未熟性であり，在胎週数が早いほど，出生時体重が少ないほど重篤である[10,11]．

網膜症では，vascular endothelial growth factorなどの血管新生因子が網膜無血管領域から放出されて血管新生をおこすと考えられている[12,13]．活動期に行われる光凝固や冷凍凝固治療は，この血管新生因子の産生と放出を抑えることが目的である．

酸素投与は網膜症発生の直接の原因ではないが，悪化させる要因である．初期の酸素投与に関する研究で，4週間高濃度酸素にさらされると網膜症の発生率が非常に増加することが示され，以後は酸素投与の厳重な管理や，抗酸化薬の外用，酸素フリーラジカル形成を促す光の曝露からの遮蔽などが行われてきた．しかし，これらの予防法十分な解決策にはならなかった．経皮膚的に連続計測して酸素をコントロールしても網膜症の発生率や重症化を抑えることはできず，ビタミンE投与などによる酸化予防の試みでも，効果に一致した意見はみられない[10,11]．

その他に，呼吸窮迫症候群，交換輸血，敗血症，脳室内出血，栄養や水分投与のアンバランスなど，呼吸や全身環境の異常に関与して網膜症を悪化させる因子として指摘されている[10,11]．

眼底検査

眼底検査の開始時期については，米国で行われた冷凍凝固の多施設共同研究（CRYO-ROP Study）では出生体重1,300 g以下，あるいは1,800 g以下で補助的に酸素投与を行った低出生体重児には，すべてスクリーニング検査を行うことをすすめている．そして出生後7～9週に最初の検査を行えば活動性を有するものの，まだ重症に至っていない網膜症の大部分を発見することができると考えられている[11]．普通は出生後4～9週に初回検査が行われていることが多い．

われわれは，在胎36週未満，出生体重が1,800 g以下，あるいは高濃度酸素使用，手術を

図8　新生児病棟での眼底検査

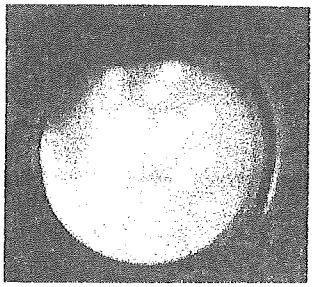

図9　倒像鏡アルゴンレーザーによる凝固斑

行った場合をすべて検査対象としている．これは軽度の網膜血管成長不全をも把握するためで，米国の基準より対象を広めにとっている．検査開始時期は，超未熟児の出生が増加していることからも，全身状態が安定したら，ただちに，遅くとも出生後3週あるいは修正在胎30週前には初回検査を行う．

眼底検査は新生児病棟で行う．眼科医のほかに，患児を抑制する者と，全身状態を観察する新生児科医師の2名の介助が必要である（図8）．

治　療

1．光凝固と冷凍凝固

網膜症が発症しても，厚生省新分類3期初期，あるいは国際分類stage 2までならば自然寛解し，視力予後もよい．しかし，さらに進行すれば網膜凝固を行う．これは無血管領域に汎凝固を行って血管新生因子の産生を抑制し，あわせて新生血管の増殖の場をなくして，網膜剝離発生の可能性を減少させることが目的である．わが国では早くから光凝固が行われており，良好な結果が得られている[12]．米国では，はるかに遅れて，まずCRYO-ROP Studyによって冷凍凝固の有用性が証明され[13]，最近になって光凝固が行われるようになってきた[15]．しかも，米国のCRYO-ROP Studyでは失明予防を目的としているのに比べて，日本ではわずかな瘢痕すらもおこさず，有用な視力を確保することを目的としており，凝固治療の時期が早い傾向にある．

光凝固はキセノンあるいはアルゴン/半導体レーザーによって行う．前者に比して後者のほうが効果は弱いが，古いキセノン光凝固装置をもっている病院はごくわずかとなっている．治療後はできるだけ頻回に眼底検査を行い，不足であれば凝固を追加する（図9）．冷凍凝固は術中の眼球障害のみならず，無呼吸発作や徐脈，血圧低下などの全身合併症をおこす危険性が高い．しかも凝固能が強いので，進行例では後に凝固縁に網膜裂孔を形成し，後の硝子体手術の予後を悪くする．

2．網膜剝離に対する治療（バックリングと硝子体手術）

網膜症がさらに進行して網膜剝離に至った場合，恒久的な視力障害をおこす．これに対しては，まず強膜バックリング手術[16][17]，ついで硝子体手術[18][19]が行われる．バックリングは眼球の外にシリコンスポンジを縫いつけて眼球壁に陥入させ，牽引を軽減させて網膜剝離を治す方法である．しかし，おもに部分網膜剝離に対して行われ，全剝離に向かえば硝子体手術が必要となる．これは，眼内に小さい器具を挿入して網膜を牽引している増殖膜（瘢痕化した新生血管由来の膜組織）を除去し，網膜剝離を治す方法である（図10，11）．

しかし，成人の網膜剝離と比べて非常に重篤なので治癒率は十分とはいえない．しかも，網膜の障害が非常に強いので，剝離が治っても視

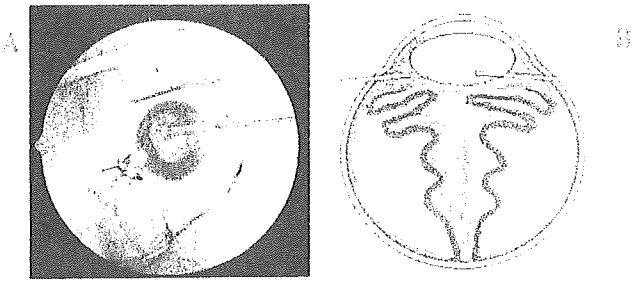

図10　硝子体手術
A：眼球前方の写真，B：眼球シェーマ

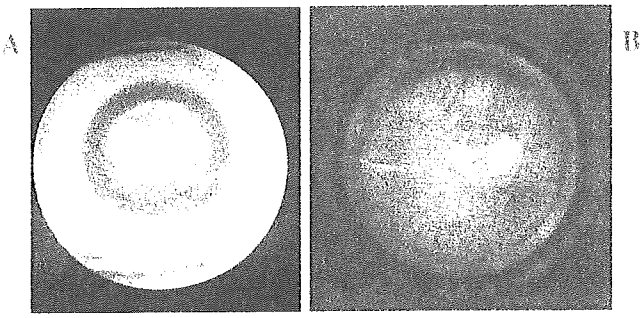

図11　硝子体手術前後
A：術前白色瞳孔の眼球前方写真
B：術後網膜が複位した眼底写真

力は光覚，手動弁程度しか得られないことも多い．硝子体手術は原則として，両眼に高度の剝離がある場合に，片眼ずつ行う．他眼が良好の視力が期待できる場合は，手術はすすめられない．たとえ光覚が得られてもその眼を使わないし，眼球の発育が悪ければ，将来は整容上から義眼を装用することになるからである．

手術時期は，網膜を復位させて視力発達を促すためには早期のほうが望ましいが，本症は手術を急ぐことはむしろ危険である．増殖膜内の血管の活動性が高く，術中に大出血をおこすと止血は不可能なので，瘢痕化が進んで増殖膜中の血管が十分に退縮するのを待ってから手術を行う．通常は網膜剝離がおこってから1～2カ月は待つことが多い．全身麻酔をかけられるか否かも大きな問題である．呼吸器も未熟で，麻酔はかけられても術後に抜管できず長期に呼吸管理をしなければならないこともある．新生児科や麻酔科と十分に相談して手術適応を決める．

晩期合併症に対する検査

光凝固後であれ自然寛解であれ，活動期を乗り切って網膜症が瘢痕化しても，眼底検査を定期的に行わなければならない．晩期合併症として，裂孔原性網膜剝離がおこる危険性がある．瘢痕が軽度であれば10歳代後半におこりやすいが，網膜ひだなど高度な牽引があれば，学童期でも裂孔が生ずる．ことに年少では片眼の視力低下に気づかないので，3～4カ月ごとに眼底検査を行い，眼球を打撲した場合は早期に受診するよう家族にすすめておく．

家族に対する説明とインフォームド・コンセント，ハビリテーション

未熟児網膜症は軽度であれば寛解するが，進行すれば失明につながることもあり，発生初期には予後がわからないことも多い．したがって家族に十分な説明を行っておくことが必要である．網膜症による視覚障害では，米国はもとより，わが国でも多数の訴訟がおこされており，医師は患児の治療のみならず，社会的な問題にも配慮しなければならない．通常，初回の眼底検査の際に，家族に未熟児網膜症の一般について説明し，現在の患児がどの状態にあるかを告げておくべきである．急に光凝固が必要になっても，すでに十分な説明がされていれば家族の納得がただちに得られる．硝子体手術のような予後が十分でない治療を行う場合は，ことにインフォームド・コンセントが重要である．

また，網膜の状態に応じて，できる限り視力を発達させるように努力するべきである．比較的視力が望めるのであれば屈折矯正や訓練などを積極的に行う．不幸にして視覚障害が重篤な場合には，日常生活や就学指導など種々の社会的問題が生ずる．発達遅滞などの重複障害も多

いので，ハビリテーションは専門家との連携のもとにできるだけを早期から行ったほうがよい．

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乳頭周囲ぶどう腫の光干渉断層計像

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要約 乳頭周囲ぶどう腫6眼6症例を検索した。年齢は6～18歳で，視力は0.04から1.5の範囲にあった。眼軸長は23～25 mmで，乳頭陥凹の深さは2～4 mmであった。光干渉断層計（OCT）では，全例で陥凹内に薄い感覚網膜があり，層構造の乱れがあった。網膜色素上皮には部分的な欠損ないし肥厚があった。従来の病理報告では，陥凹内の網膜は，朝顔症候群では肥厚し，乳頭コロボーマでは非薄であるとされている。今回のOCT所見は，乳頭周囲ぶどう腫が朝顔症候群よりもコロボーマに近いことを示している。

Optical coherence tomographic findings of peripapillary staphyloma

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Abstract. We observed peripapillary staphyloma in 6 eyes of 6 patients. Their ages ranged from 6 to 18 years. Their visual acuity ranged from 0.04 to 1.5. The axial length was between 23 mm to 25 mm. The depth of the disc excavation was between 2 to 4 mm. Optical coherence tomography (OCT) showed, in all the eyes, the presence of sensory retina with irregular layer structure in the area of staphyloma. The retinal pigment epithelium showed partial hypo-or hyperplasia. Previous histopathological studies show that the sensory retina in the staphylomatous area is thick in morning glory syndrome and is thin in coloboma of the optic disc. The present OCT findings show that peripapillary staphyloma is more similar to disc coloboma rather than to morning glory syndrome.

Rinsho Ganka (Jpn J Clin Ophthalmol) 58(7): 1241-1243, 2004

緒 言

乳頭周囲ぶどう腫は，乳頭領域が拡大陥凹し，その底に正常，ないしはそれに近い乳頭が存在するきわめて稀な乳頭部先天異常である[1]。過去の乳頭部先天異常の病理報告は，乳頭コロボーマ[2～4]が多く，朝顔症候群と類縁疾患はわずか[5～7]に過ぎず，乳頭周囲ぶどう腫に関する報告はない。

近年，光干渉断層計（optical coherence tomography：OCT）が開発され網膜や網膜色素上皮の生体観察が可能になった。今回筆者らは乳頭周囲ぶどう腫の乳頭陥凹部の網膜をOCTで観察したので報告する。

対象と方法

乳頭周囲ぶどう腫6例6眼（男児2例，女児4例，年齢6～18歳）を対象とした。視力，眼底検査，超音波断層検査（NIDEK US-2500 Echo Scan）とともに光干渉断層計（ZEISS Systems Optical Coherence Tomography Model 2010, OCT2）による測定を施行した。OCTは，乳頭陥凹部を中心にscan length 5 mmもしくは7 mmにてスキャンした。

結 果

表1に示すように，視力は0.04～1.5とさまざまであった。黄斑形成を認めた5眼は0.1以上の

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表 1　6 症例の OCT を含む検査所見

症例	年齢（歳）	性別	患側	視力	眼底所見			エコー所見	OCT 所見	
					黄斑形成	乳頭周囲網脈絡膜変性	乳頭上白色組織	陥凹の深さ（mm）	感覚網膜	網膜色素上皮
1	6	男	左	1.5	＋	＋	－	2	層構造の乱れ	菲薄
2	12	男	右	0.3	＋	＋	－	3	層構造の乱れ（一部肥厚）	菲薄
3	8	女	右	0.04	－	＋	－	3	層構造の乱れ	菲薄（一部肥厚）
4	7	女	右	0.6	＋	＋	－	4	層構造の乱れ（一部肥厚）	菲薄（一部肥厚）
5	18	女	左	0.5	＋	＋	＋	3	層構造の乱れ	菲薄
6	8	女	右	1.0	＋	＋	－	3	層構造の乱れ	菲薄

視力を有したが，1 眼は陥凹周囲の輪状網脈絡膜萎縮が黄斑まで及び，視力不良であった。陥凹部に軽度の白色組織を認めたものは 1 例のみ（症例 5）で，初診時（生後 4 か月）陥凹底に正常に近い乳頭があったが，経過観察中（8 歳）に白色組織が出現し，乳頭の輪郭が不明瞭となった。

エコー所見は，6 例すべての乳頭部に浅い陥凹があり，深さは，2～4 mm であった。いずれもその後方に正常に近い視神経像を認めた。眼軸長は，23～25 mm であった。

OCT 所見として，網膜断層像を感覚網膜と網膜色素上皮（retinal pigment epithelium：RPE）を観察した。6 例全例に陥凹内に薄い感覚網膜が存在したが，層構造に乱れがあった。2 例はごく一部にだけ肥厚を認めた。また RPE は，すべての症例で全体は菲薄であったが，部分的な欠損やむしろ肥厚もみられた。

典型的な症例（症例 1）として 6 歳，男児の例を示す（図 1～3）。

考按

コロボーマの病因が胎生裂閉鎖不全（胎生 4～5 週）[8,9]であることは周知の事実である。一方朝顔症候群と乳頭周囲ぶどう腫の病因は，胎生裂閉鎖不全あるいは後部強膜の発生異常（胎生 20 週頃）と考えられている[1,10～12]が，近年の研究では胎生裂閉鎖不全が支持されることが多い[13,14]。乳頭の形態から，コロボーマ，乳頭周囲ぶどう腫および朝顔症候群は，乳頭部先天異常の疾患のなかで同一スペクトルム上にあり[15,16]，発生機転の程度や時期の差によって表現型が多彩になると考えられている。

病理組織所見については，1972 年に Willis ら[4]がコロボーマは視神経の萎縮がみられ，篩状板は通常より後方に位置しているか欠損しており，異形成網膜とグリア組織が陥凹内にみられたと報告している。一方，過去の朝顔症候群と類縁疾患の病理報告は数例[5～7]に過ぎない。1961 年に Pedler[5]は，朝顔症候群と思われる病理所見において，乳頭上のグリア組織に牽引されて一塊となった異形成網膜，篩状板の後方移動を報告している。これは，陥凹内の白色組織によって網膜が陥凹内に牽引され異形成となって，肥厚したと考えられる[17]。

今回の OCT 像では，表 1 のごとく 6 例いずれも感覚網膜の層構造が乱れていた。また網膜と RPE ともに一部は肥厚を認めるものの，全般には菲薄であった。この結果を乳頭コロボーマと朝顔症候群の病理組織所見と比較検討すると，乳頭周囲ぶどう腫の組織像はコロボーマに近いと考えられる。

しかし，乳頭周囲ぶどう腫の陥凹の深さについて過去の報告[10,11]では平均 3～8 mm で，9.77 mm の症例も報告されている[18]。これらと比較すると，今回の対象例（2～4 mm）は陥凹が浅い軽症例に相当する。陥凹部が深く，その周囲の網脈絡膜萎縮が広汎であれば，黄斑の形成が不良で，固視が悪く，OCT 検査が十分にできない。したがって，今回 OCT が測定できたのは軽症例に限られた。それでも陥凹網膜の菲薄化があったので，陥凹が深ければ網膜はより牽引されて薄くなると考えられる。

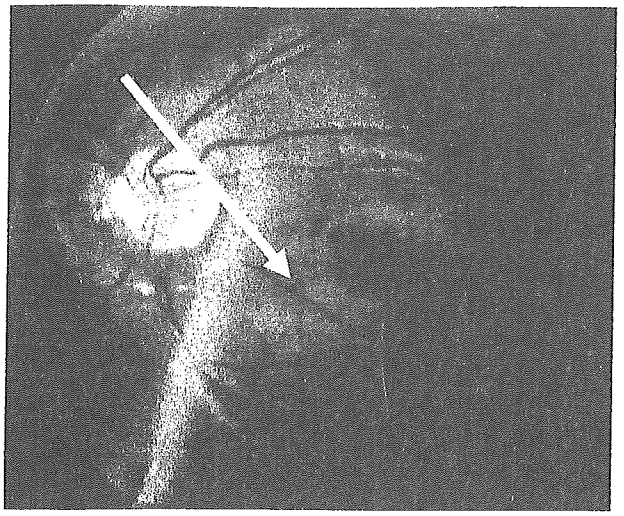

図 1　6 歳男児（症例 1）の左眼眼底写真
左眼視力（1.5×＋0.5D）で浅い陥凹を認め，乳頭上の白色組織はなかった。矢印は OCT のスキャンした部位を示す。

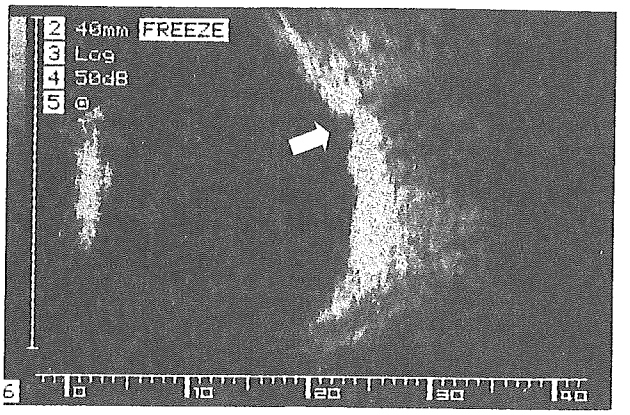

図 2　症例 1 の超音波断層所見
乳頭周囲に浅い陥凹（2 mm，矢印）を認め，眼軸長は 23 mm であった。

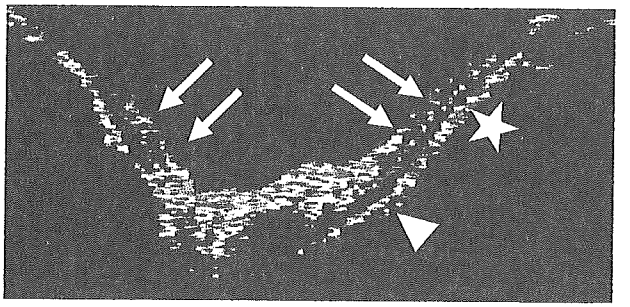

図 3　症例 1 の OCT 所見
感覚網膜の層構造の乱れ（矢印），RPE の菲薄（矢頭），および一部肥厚（星印）所見を認めた。

また，乳頭周囲ぶどう腫のなかでは，症例 5 のように陥凹部に軽微な乳頭上の白色組織を認めることもある。これは朝顔症候群の軽症例にあたると思われるが，乳頭上の白色組織が網膜を牽引して一塊とならない限り，網膜は陥凹形成に伴って菲薄に向かうと思われる。

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感覚器障害研究事業

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Clinical Report

Microdeletion in the *SHOX* 3′ Region Associated With Skeletal Phenotypes of Langer Mesomelic Dysplasia in a 45,X/46,X,r(X) Infant and Leri–Weill Dyschondrosteosis in her 46,XX Mother: Implication for the *SHOX* Enhancer

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It is known that *SHOX* nullizygosity results in Langer mesomelic dysplasia (LMD) and *SHOX* haploinsufficiency leads to Leri–Weill dyschondrosteosis (LWDC). Here, we report on a microdeletion in the *SHOX* 3′ region identified in a Japanese infant with LMD-compatible skeletal features and a 45,X[191]/46,X,r(X)(p22.3q24)[9] karyotype and in her mother with LWDC-compatible skeletal features and a normal 46,XX karyotype. Physical and auxological examinations revealed mesomelic appearance, ulnarly deviated hands, and borderline micrognathia in the infant, and relatively short forearms and lower legs in the mother. Radiological studies indicated mesomelia, markedly curved radii, hypoplastic ulnas and fibulas, and metaphyseal splaying in the infant, and borderline to mild curvature of the radii, decreased carpal angles, and high-normal triangularization index in the mother. Cytogenetic and molecular studies showed that the ring X chromosome of the infant was missing *SHOX* and of paternal origin, whereas the cytogenetically normal X chromosomes of the infant and one of the two X chromosomes of the mother, though they retained *SHOX* with normal coding sequences, had a microdeletion in the *SHOX* 3′ region. The microdeletion started from a position ~200 kb from *SHOX* coding sequences, and spanned 240–350 kb in physical length involving *DXYS233*. The results, in conjunction with those reported by Flanagan et al. [2002], suggest that a *cis*-acting enhancer exists in the *SHOX* 3′ region around *DXYS233*.

KEY WORDS: *SHOX*; Langer mesomelic dysplasia; Leri–Weill dyschondrosteosis; enhancer; 3′ deletion

INTRODUCTION

Short stature homeobox containing gene (*SHOX*) cloned from the short arm pseudoautosomal region of the X and the Y chromosome is a transcription factor gene exclusively expressed in the developing skeletal tissues of distal limbs and pharyngeal arches [Rao et al., 1997; Clement-Jones et al., 2000]. *SHOX* haploinsufficiency results in short stature, Turner skeletal features, and Leri–Weill dyschondrosteosis (LWDC) [Ogata, 2002], and *SHOX* nullizygosity leads to Langer mesomelic dysplasia (LMD) [Ogata et al., 2002]. However, ~20% of patients with LWDC have no abnormality in the *SHOX* coding sequences [Ogata, 2002], and two sib patients with LMD have a single normal *SHOX* coding sequence [Zinn et al., 2002]. In this context, Flanagan et al. [2002] have described a large family in which seven patients with two *SHOX* genes accompanied by intact coding sequences have an association of LWDC phenotype with apparent hemizygosity for a region encompassing *DXYS233* at a 3′ position ~300 kb from *SHOX*. Although the results of *DXYS233* microsatellite analysis might be due to amplification failure caused by a polymorphism in the sequence for the primer hybridization, they also demonstrated a monoallelic *SHOX* expression in the bone marrow fibroblasts taken from the distal radius of the proband. Thus, the results suggest that an enhancer for *SHOX* expression exists in a region around *DXYS233*, and that loss of the enhancer results in the development of LWDC.

Here, we report on an infant with LMD-compatible skeletal phenotype and a mosaic ring X chromosome and her mother with LWDC-compatible skeletal phenotype and a normal karyotype. Molecular studies showed a microdeletion in the *SHOX* 3′ region on the cytogenetically normal X chromosome transmitted from the mother to the infant, providing further support for the presence of a *SHOX* enhancer in this region.

CLINICAL REPORT

This Japanese female infant was born at 39 weeks of gestation after an uncomplicated pregnancy and delivery. At birth, her length was 50.0 cm (+0.8 SD) and weight 3.73 kg (+2.2 SD). At 7 months of age, she was referred to us because of mesomelic appearance, ulnarly deviated hands, and borderline micrognathia (Fig. 1A). Except for the borderline micrognathia, she had no Turner somatic stigmata such as cubitus valgus, short metacarpals, webbed neck, ear abnormalities, or ptosis. Bone survey showed LMD-compatible skeletal findings such as severely shortened distal limb bones, markedly curved radii, hypoplastic ulnas and fibulas, and metaphyseal splaying (Fig. 1B). Ultrasound studies delineated no cardiac or renal abnormalities. On the latest examination at 1.5 years, her

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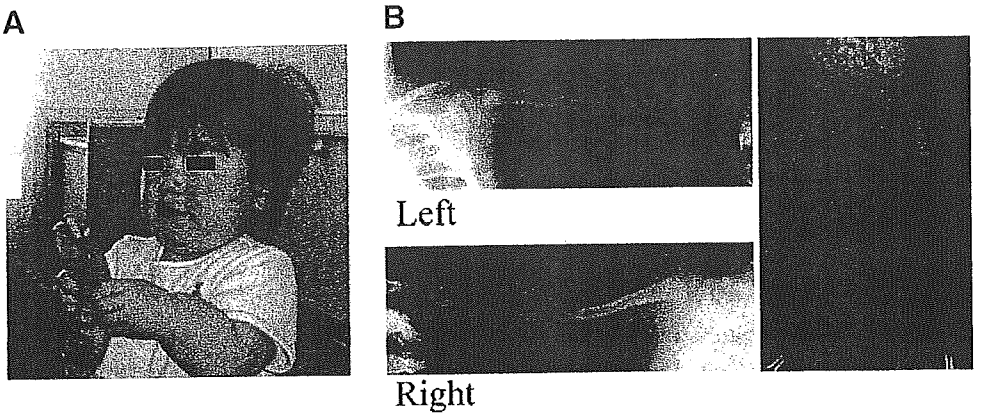

Fig. 1. A: Photograph of the infant at 1.5 years of age. B: Roentgenograms of the infant at 7 months of age.

developmental milestones were normal, and her length was 76.2 cm (−1.8 SD), weight 10.8 kg (+0.5 SD), head circumference 45.7 cm (−0.4 SD), sitting height 49.0 cm (no reference data), and arm span 46.0 cm (no reference data).

The 30-year-old mother was initially regarded as having an apparently normal phenotype with no mesomelic appearance, wrist deformity, or Turner skeletal features. She was 158.1 cm in height (±0 SD) and 53.2 kg in weight (±0 SD), and had no muscular hypertrophy. However, her wrist movement appeared to be somewhat limited. Thus, auxological studies were performed as described by Cameron [1987], revealing relatively short forearms and lower legs, as well as large hands (Fig. 2A). Furthermore, radiological studies of the hands and forearms showed borderline to mild radial curvature, decreased carpal angles (right 101°, left 106°) (normal range, >118°) [Kosowics, 1965], and high-normal triangularization index (right 3.4, left 3.6) (normal range, 1.8–3.7) [Binder et al., 2001] (Fig. 2-B). Thus, she was assessed as having mild LWDC-compatible skeletal phenotype. Allegedly, her 63-year-old father was 162 cm tall (−0.6 SD for his age), her 60-year-old mother 163 cm tall (+1.8 SD for her age), and her 33-year-old sister 158.0 cm tall (±0 SD), with no obviously abnormal

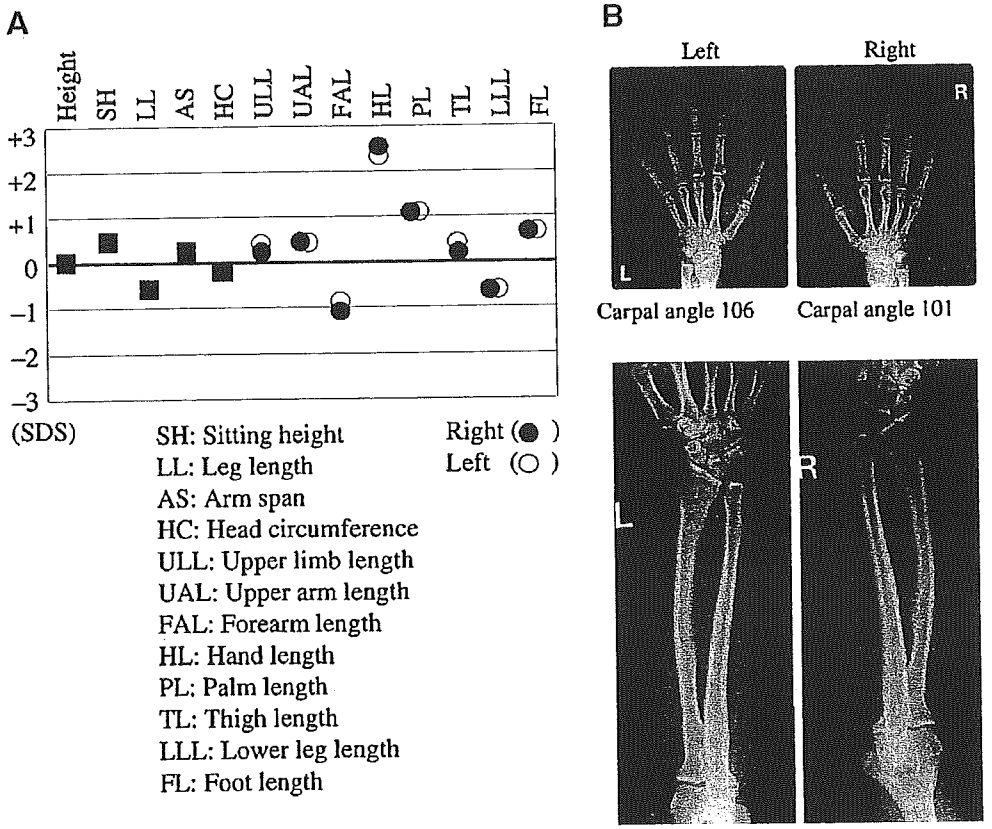

Fig. 2. A: auxological data of the mother. Each measurement is based on the methods described by Cameron [1987], and the auxological data have been assessed by the Japanese body size data (1992–1994) (Research Institute of Human Engineering for Quality of Life, http://www.hql.jp). B: Roentgenograms of the mother at 30 years of age.

findings. Since her parents and sister lived in a local city far away from Tokyo, detailed examinations including auxological and radiological studies were not performed. The 35-year-old father was 178.3 cm in height (+1.4 SD), and clinically normal.

CYTOGENETIC AND MOLECULAR STUDIES

This study has been approved by the Institutional Review Board Committee at National Center for Child Health and Development. After taking written informed consent, peripheral blood samples were obtained from the infant and the parents.

G-banding chromosome analysis was carried out for peripheral lymphocytes, showing a 45,X[191]/46,X,r(X)(p22.3q24)[9] karyotype in the infant, a 46,XX[50] karyotype in the mother, and a 46,XY[50] karyotype in the father. Fluorescence *in situ* hybridization (FISH) analysis was performed for *SHOX* and other four loci as described previously [Ogata et al., 2001a] using *DXZ1* as an internal signal control, demonstrating loss of *SHOX* from the ring X chromosome and determining the breakpoints between *DAX1* and *KAL1* and between *GRIA3* and *GPC3* (Fig. 3A,B). *SHOX* was normally present on the cytogenetically normal X chromosome of the infant and on the two X chromosomes of the mother, as well as on the X and the Y chromosomes of the father. Furthermore, microsatellite analysis was carried out for *SHOX*-5′UTR-CA as described previously [Belin et al., 1998], indicating the paternal origin of the ring X chromosome: the PCR product size was 151 bp in the infant and the mother and 141 and 153 bp in the father. Direct sequencing was performed for *SHOX* coding exons and their flanking introns by the previously described method [Shears et al., 1998], showing no mutation in the infant and the parents.

Thus, a possible deletion at the *SHOX* 3′ region around *DXYS233* was examined for the cytogenetically normal X chromosome of the infant and the mother. PCR deletion analysis was carried out for eight loci/regions with primers reported in Genome Database (http://www.gdb.org/) or designed by us (available on request) using *SHOX* exon 2 as an internal control, identifying a 240–350 kb deletion with the breakpoints between *rs5946324* and *rs5988437* and between *rs4468091* and *RH65317* in the infant (Fig. 3A,C). Furthermore, FISH analysis was carried out with an RP11-309M23 probe (Ensemble Database, http://www.ensembl.org/) that defines a chromosomal region deleted in this infant, showing no signal in the infant and only a single signal in the mother (Fig. 3A,B). The results of the father were normal.

After consultation, the mother decided not to inform the maternal parents and the sister of the results. Thus, together with their residence apart from Tokyo, it was impossible to determine whether the microdeletion was produced as a *de novo* event or transmitted from either of the maternal

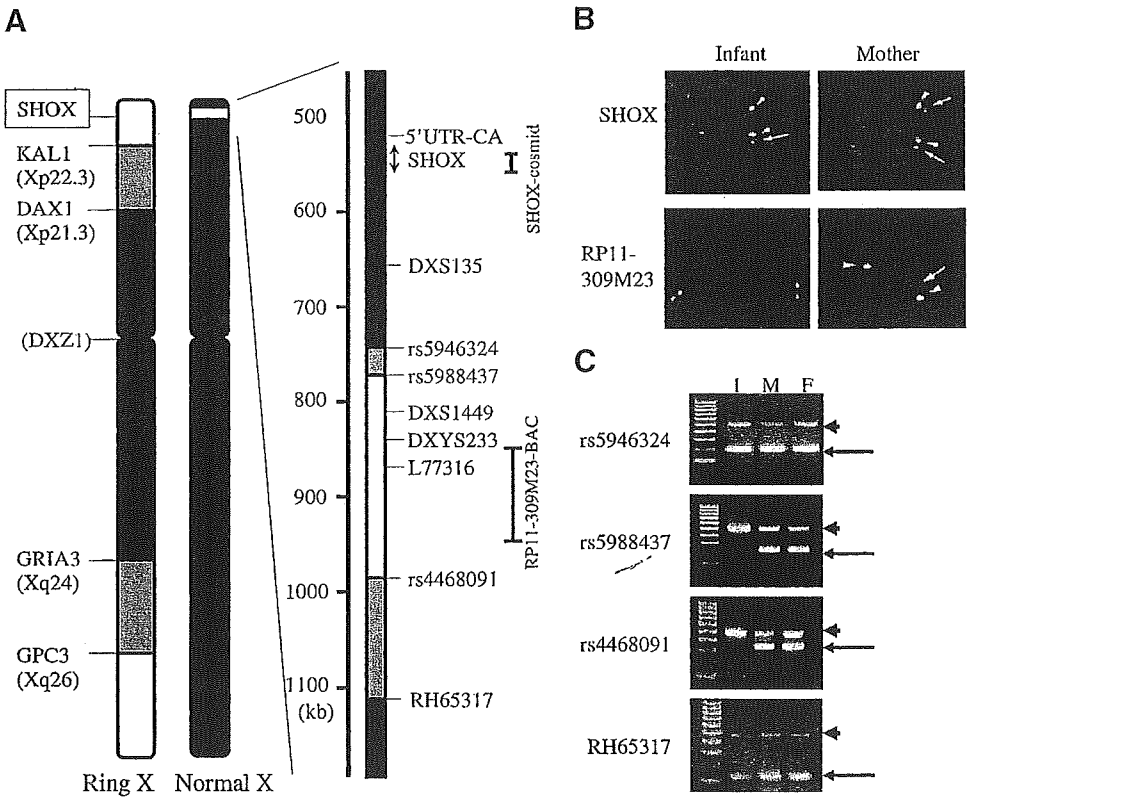

Fig. 3. **A**: Summary of the molecular studies. The black and the white areas denote the preserved and the deleted regions, respectively. The gray areas depict the regions where the breakpoints should exist. For the ring X chromosome, the deleted regions have been determined by FISH analysis for the five loci shown in the left side. For the cytogenetically normal X chromosome, the microdeletion has been demonstrated by PCR deletion analysis for eight loci/regions shown on the right side. The two vertical bars indicate the regions defined by FISH analysis with a SHOX-cosmid probe and an RP11-309M23-BAC probe, respectively. The Arabic numbers represent the physical length from the Xp/Yp telomere (kb). **B**: Representative results of the FISH analysis. The arrows indicate the *SHOX* or the RP11-309M23 region, and the arrowheads represent *DXZ1*. *SHOX* is deleted from the ring X chromosome of the infant, and is normally preserved on the cytogenetically normal X chromosome of the infant as well as on the maternal X chromosomes. The RP11-309M23 region is lost from the cytogenetically normal X chromosome as well as from the ring X chromosome of the infant, and from one of the two X chromosomes of the mother. **C**: Representative results of the PCR deletion analysis. I: infant; M: mother; and F: father. In each PCR analysis, the target sequence (arrows) and the *SHOX* exon 2 (arrowheads) have been amplified concomitantly. The cytogenetically normal X chromosome of the infant is positive for *rs5946324* and *RH65317* and negative for *rs5988437* and *rs4468091*.

parents, especially from the maternal father with a low-normal height, and whether the microdeletion was present or absent in the maternal sister with an average height.

DISCUSSION

The present study showed LMD-compatible skeletal phenotype in an infant with a mosaic ring X chromosome and mild LWDC-compatible skeletal phenotype in her mother with a normal karyotype. Furthermore, it was shown that the ring X chromosome of the infant was missing *SHOX* and of paternal origin, and that the cytogenetically normal X chromosomes of the infant and one of the two X chromosomes of the mother, though they retained *SHOX* with normal coding sequences, had a 240–350 kb microdeletion in the *SHOX* 3′ region.

The results would provide further support for the presence of a *cis*-acting enhancer for *SHOX* in the 3′ region of the gene. In this regard, it is notable that the microdeletion of the infant and the mother encompasses *DXYS233*, because one allele of *DXYS233* was not amplified in the seven familial LWDC patients with two *SHOX* genes accompanied by intact coding sequences described by Flanagan et al. [2002]. Thus, although loss of *DXYS233* was not directly demonstrated in the seven patients, it is inferred that a microdeletion encompassing *DXYS233* also exists in the seven patients, and that the putative enhancer resides in an overlapping deleted region around *DXYS233*. In support of the presence of such an enhancer at a position 200–550 kb from *SHOX* coding sequences, it has been reported in several genes that a deletion or a disruption in a 5′ or 3′ region at a position 10–1,000 kb from the gene coding sequences can affect the function of the corresponding gene [Kleinjan and van Heyningen, 1998]. In this context, two sib patients with typical LMD have apparently one intact allele of *SHOX* (Table I, cases 15 and 16), and ~20% of LWDC patients have no demonstrable *SHOX* abnormalities [Ogata, 2002]. Such patients would be worth analyzing for the putative enhancer region around *DXYS233*.

Clinical features appear to be mild in the infant and the mother. First, the statural growth was well preserved in the infant and the mother. Although the fairly sustained statural growth of the infant and the mother would partly be contributed by the high genetic growth potential as suggested by the relative tall heights of the father and the maternal mother, the previously reported LMD patients with *SHOX* abnormalities have extreme short stature (Table I), and Japanese female patients with *SHOX* haploinsufficiency and normal ovarian function have severe short stature (−2.6 ± 0.8 SD) [Fukami et al., 2004]. Second, while LMD of the infant would be fairly typical, LWDC of the mother was obviously mild as compared with that of adult females with *SHOX* haploinsufficiency [Ogata, 2002; Munns et al., 2003]. In this regard, clinical spectrum in patients with LMD appears to be somewhat variable in terms of the presence or absence of common Turner skeletal features (Table I), and that in patients with *SHOX* haploinsufficiency is known to be variable from borderline short stature only phenotype to severe LWDC phenotype with and without common Turner skeletal features [Ogata, 2002; Munns et al., 2003]. Furthermore, clinical phenotype in patients with a deletion or a disruption affecting a *cis*-acting enhancer for several disease genes has also been reported to range widely from apparently normal phenotype to typical disease phenotype [Kleinjan and van Heyningen, 1998]. Thus, it remains to be elucidated whether the apparently mild phenotypes of the infant and the mother are characteristic of the deletion of the putative enhancer for *SHOX*.

For the skeletal features of the infant and the mother, other possibilities remain tenable at present. First, a hidden

TABLE I. Summary of Patients With Langer Mesomelic Dysplasia

Patients			Clinical features		Mutations			
Case	Age	Sex	Height SDS	Other features[a]	Patient	Father	Mother	Reference
1	Fetus	F	—	N.D.	Deletion/Deletion	(−)/(−)	Deletion/(−)	Belin et al. [1998]
2	Fetus	N.E.	—	N.D.	Deletion/Deletion	Deletion/(−)	Deletion/(−)	Shears et al. [1998]
3	12 years	M	−7.2	None	Deletion/Deletion	Deletion/(−)	Deletion/(−)	Robertson et al. [2000]
4	84 years	F	−5.5	HP, S4M, CV	R153C/V163F	N.E.	N.E.	Zinn et al. [2002]
5	7 years	F	−5.5	HP, CV	Deletion/ R118fsX130	R118fsX130/(−)	Deletion/(−)	Zinn et al. [2002]
6	65 years	M	−7.2	HP, CV	P243fsX321 (homo/hemi)[b]	N.E.	N.E.	Zinn et al. [2002]
7[c]	24 years	F	−8.9	N.D.	R173C/R173C	N.E.	R173C/(−)	Shears et al. [2002]
8[c]	Neonate	M	—	MG?	R173C/R173C	R173C/(−)	R173C/R173C	Shears et al. [2002]
9	1.5 years	M	−4.2	None	Deletion/R168W	Deletion/(−)	R168W/(−)	Ogata et al. [2002]
10	Fetus	F	—	None	Deletion/Deletion	(−)/(−)	Deletion/(−)	Thomas et al. [2004]
11[d]	Adult	M	N.D.	N.D.	A170P/A170P	A170P/(−)	A170P/(−)	Sabherwal et al. [2005]
12[d]	38 years	F	−8.5	N.D.	A170P/A170P	A170P/(−)	A170P/(−)	Sabherwal et al. [2005]
13[d]	Adult	F	N.D.	N.D.	A170P/A170P	A170P/(−)	A170P/(−)	Sabherwal et al. [2005]
14[d]	2 years	F	N.D.	N.D.	A170P/A170P	A170P/(−)	A170P/A170P	Sabherwal et al. [2005]
15[e]	35 years	F	−6.8	HP, CV	Deletion/(−)	N.E.	N.E.	Zinn et al. [2002]
16[e]	33 years	M	−6.2	HP, S4M, CV	Deletion/(−)	N.E.	N.E.	Zinn et al. [2002]
17	1.5 years	F	−1.8	MG?	Deletion/ 3′ deletion	(−)/(−)	3′ deletion/(−)	This report

The (−) symbol indicates the absence of a recognizable mutation. SDS, standard deviation score; F, female; M, male; N.E., not examined; N.D., not described; HP, high arched palate; S4M, short 4th metacarpals; CV, cubitus valgus; and MG, micrognathia.

[a]Except for severe mesomelia and skeletal deformities in the forearms and shanks.

[b]Case 6 is homozygous or homozygous for P243fsX321.

[c]Case 7 is the mother of case 8, and her consanguineous husband has Leri–Weill dyschondrosteosis and the same SHOX mutation.

[d]Cases 11, 12, and 13 are siblings; case 13 is the mother of case 14, and her consanguineous husband has Leri–Weill dyschondrosteosis and the same SHOX mutation.

[e]Case 15 and case 16 are siblings.

mutation might exist in the unexamined regions such as the intron or the promoter sequences of *SHOX* on the cytogenetically normal X chromosome transmitted from the mother to the infant. Second, a different gene(s) involved in the skeletal development of distal limbs might be affected in the infant and the mother. In this regard, Ventruto et al. [1983] have reported a two-generation family in which LWDC-like phenotype is cosegregated with t(2;8)(q31;p21), and the translocation breakpoint has been located at a position ~60 kb telomeric to *HOXD* gene cluster [Spitz et al., 2002]. Furthermore, Kantaputra et al. [1992] have described a large family with an autosomal dominant form of mesomelic dysplasia, and the gene has been mapped to a 2q24–32 region encompassing *HOXD* gene cluster [Fujimoto et al., 1998]. However, loss of *HOXD9–HOXD13* expressed in the limb region has been reported to cause synpolydactyly rather than mesomelic dysplasia [Goodman et al., 2002], so that the relevance of *HOXD* genes to the development of mesomelic dysplasia remains to be clarified, as well as a possible role of the putative global control region for the expression of multiple *HOXD* and other neighboring genes [Spitz et al., 2003].

Further two matters would also be worth pointing out in the present study. First, except for the borderline micrognathia, the infant had no Turner somatic or visceral features in the presence of the mosaic ring X chromosome. This would not be surprising, because clinical features are highly variable in Turner syndrome [Ogata and Matsuo, 1995]. In addition, the ring X chromosome should retain the putative lymphogenic gene(s) between *DMD* and *MAOA* responsible for the development of soft tissue and visceral features [Ogata et al., 2001b], and should be present more frequently in the slowly dividing target tissues for the soft tissue and visceral features than in the rapidly dividing lymphocytes utilized for the karyotype analysis. This would also be relevant to the lack of such features in this infant. Second, the mother had relative large hands. Since this phenotype has also been reported in Turner patients [Gravholt and Weis Naeraa, 1997], it may be characteristic of *SHOX* haploinsufficiency.

In summary, the results, in conjunction with those reported by Flanagan et al. [2002], argue for the presence of a *cis*-acting enhancer in the *SHOX* 3′ region around *DXYS233*. Further studies will permit a definite conclusion on this matter.

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Segmental and Full Paternal Isodisomy for Chromosome 14 in Three Patients: Narrowing the Critical Region and Implication for the Clinical Features

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We report on segmental and full paternal isodisomy for chromosome 14 in three previously unreported Japanese patients. Patient 1 was a 5$^{6}/_{12}$-year-old girl, Patient 2 was a male neonate, and Patient 3 was a 6$^{7}/_{12}$-year-old girl. Physical examination at birth showed various somatic features characteristic of paternal uniparental disomy for chromosome 14 (upd(14)pat) such as hairy forehead, protruding philtrum, micrognathia, small thorax, and abdominal wall defects in Patients 1–3, and the constellation of somatic features was persistently observed in Patients 1 and 3. Radiological studies at birth delineated unique bell-shaped thorax with coat-hanger appearance of the ribs in Patients 1–3, but the thoracic deformity ameliorated in Patients 1 and 3 by mid childhood. Chromosome analysis showed a 46,XX karyotype in Patients 1 and 3 and was not performed in Patient 2. Microsatellite analysis indicated full paternal isodisomy for chromosome 14 in Patients 1 and 2 and segmental paternal isodisomy for chromosome 14 distal to *D14S981* at 14q23.3 in Patient 3. Methylation specific PCR assay for the differentially methylated region (DMR) of *GTL2* at 14q32 yielded positive products with methylated allele specific primers and no products with unmethylated allele specific primers in Patients 1–3. Since clinical phenotype was similar between Patient 3 with segmental upd(14)pat and Patients 1 and 2 with full upd(14)pat, the results are keeping with the 14q32 localized imprinted genes as the critical components of the phenotype observed in upd(14)pat and help narrow the search for additional genes to the ~40 Mb region distal to *D14S981*. Furthermore, it is likely that the characteristic thoracic deformity ameliorates with age.

KEY WORDS: paternal disomy; segmental disomy; chromosome 14; somatic features; thoracic deformity

INTRODUCTION

Paternal uniparental disomy for chromosome 14 (upd(14)pat) is associated with a distinctive constellation of clinical features such as characteristic face with blepharophimosis, prominent philtrum and micrognathia, small thorax, abdominal wall defects, and developmental retardation [Sutton and Shaffer, 2000; Chu et al., 2004]. This condition is also radiologically characterized by unique bell-shaped thorax with coat-hanger appearance of the ribs [Offiah et al., 2003]. The thoracic deformity often results in lethal respiratory failure during infancy. In addition, polyhydramnios is seen in most pregnancies with upd(14)pat.

To date, upd(14)pat has been identified in a total of 15 patients [Wang et al., 1991; Papenhausen et al., 1995; Walter et al., 1996; Cotter et al., 1997; Klein et al., 1999; Berend et al., 2000; Yano et al., 2001; Coveler et al., 2002; Kurosawa et al., 2002; McGowan et al., 2002; Offiah et al., 2003; Chu et al., 2004; Stevenson et al., 2004]. Of the 15 patients, 10 patients have Robertsonian translocations involving chromosome 14 such as t(13;14) and t(14;14), and the remaining five patients have a normal karyotype. Genotyping analysis has confirmed upd(14)pat in such patients, including a segmental isodisomy for a 14q12–14qter region in a single patient with characteristic upd(14)pat phenotype [Coveler et al., 2002]. This implies the involvement of an imprinted gene(s) in the development of upd(14)pat phenotype, and locates the major locus or loci for the upd(14)pat phenotype to the 14q12–14qter region. Consistent with this, the 14q32 segment is known to contain imprinted genes such as *DLK1*, *GTL2* (also known as *MEG3*), *PEG11*, and *MEG8* [Charlier et al., 2001; Cavaille et al., 2002]. The possibility that the clinical phenotype in upd(14)pat is contributed by the unmasking of a recessive allele(s) due to isodisomy is unlikely, because apparently full paternal heterodisomy as well as isodisomy has been found in patients with the distinct clinical phenotype [Chu et al., 2004].

However, the critical region is still large. Furthermore, since only the infantile phenotypes have been described in most patients, the long-term clinical course remains to be clarified in this condition. Here, we report three patients with upd(14)pat.

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The results further narrow down the critical region and suggest improvement of thoracic deformity with age.

MATERIALS AND METHODS

Patients

Three previously unreported Japanese patients (one male and two females) were examined in this study. Their clinical features are summarized in Table I. Patients 1–3 were born prematurely after pregnancies complicated by polyhydramnios. Patients 1 and 2 were delivered by Caesarean sections because of placental abnormalities, while Patient 3 was born by vaginal delivery. Patient 1 had severe asphyxia at birth, and was treated with endotracheal intubation and mechanical ventilation for 4 days and with oxygen and nasal directional positive airway pressure for the following 61 days at the neonatal intensive care unit (NICU). Subsequently, oxygen was supplied via a nasal mask for 355 days at the pediatric ward. Patient 2 died of respiratory failure at 2 hr of age despite an intensive care including mechanical ventilation and oxygen. Patient 3 had mild asphyxia at birth, and received oxygen in an incubator for 34 days at the NICU and via nasal mask for the following 96 days at the pediatric ward. After the discharge, Patients 1 and 3 had uneventful clinical course with no episode of respiratory infection. In addition, although pulmonary function test was not performed, they were able to play actively with peers.

Birth size appeared to be well preserved, although gestational age-matched reference data were not available in Patient 1. Childhood body size remained within the normal range in Patients 1 and 3. Moderate developmental retardation was indicated in childhood of Patients 1 and 3. Physical examination at birth revealed various somatic features consistent with upd(14)pat such as hairy forehead, protruding philtrum, micrognathia, small thorax, and abdominal wall defects in Patients 1–3, and the constellation of somatic features persisted into childhood in Patients 1 and 3. Radiological studies at birth delineated unique bell-shaped thorax with coat-hanger appearance of the ribs in Patients 1–3, but the thoracic deformity ameliorated in Patients 1 and 3 by mid childhood (Fig. 1). Parental age was variable at the time of birth of Patients 1–3 and parental heights were normal in Patients 1 and 3.

Conventional and Molecular Cytogenetic Studies

This study has been approved by the Institutional Review Board Committee at National Center for Child Health and Development. Chromosome analysis was performed on 50 peripheral lymphocytes in Patients 1 and 3. Fluorescence *in situ* hybridization (FISH) analysis was also carried out on lymphocyte metaphase spreads in Patients 1 and 3, using a ~186 kb BAC probe containing *DLK1* (RP11-566J3) and a ~165 kb BAC probe containing *GTL2*, *PEG11*, and *MEG8* (RP11-123M6) (BACPAC Resources Center, http://bacpac.chori.org/), together with a 14q telomere probe (Vysis, http://www.vysis.com/) used as an internal signal control. The RP11-566J3 and RP11-123M6 probes were labeled with digoxigenin and detected by rhodamine anti-digoxigenin, and the 14q telomere probe was detected according to the manufacture's protocol.

Microsatellite Analysis

Microsatellite analysis was performed for multiple loci on chromosome 14 (Fig. 2A). In brief, leukocyte genomic DNA of Patients 1–3 and their parents was amplified by polymerase chain reaction (PCR) with fluorescently labeled forward primers and unlabeled reverse primers, and the PCR products were determined for the fragment size on an ABI PRISM 310 autosequencer using GeneScan (Applied Biosystems, http://www.appliedbiosystems.com/). The primer sequences were as described in Genome Database (http://www.gdb.org/).

Methylation Specific PCR Assay

Methylation pattern was analyzed for the differentially methylated region (DMR) of *GTL2* where cytosines at CpG dinucleotides are methylated on the paternally derived allele and unmethylated on the maternally derived allele [Murphy et al., 2003]. In short, after bisulphite treatment with EZ DNA methylation kit (Zymo Research, http://www.zymor.com/) that converts all the cytosines except for methylated cytosines at the CpG islands into uracils and subsequently thymines, PCR amplification was performed with a pair of primers specific to the methylated allele of paternal origin and with another pair of primers specific to the unmethylated allele of maternal origin. The primer sequences and the PCR conditions were as reported previously [Murphy et al., 2003]. For a control, a DNA sample from a clinically normal individual was utilized with permission.

RESULTS

Conventional and Molecular Cytogenetic Studies

Normal female karyotype was identified in Patients 1 and 3, and two signals were detected by RP11-566J3 and RP11-123M6 as well as by the 14q telomere probe in Patients 1 and 3. Thus, the karyotype was determined as 46,XX.ish 14q32(DLK1 × 2,GTL2 × 2,PEG11 × 2,MEG8 × 2) in Patients 1 and 3.

Microsatellite Analysis

The data are summarized in Figure 2A and representative results are shown in Figure 2B. In Patients 1 and 2, single peaks only were detected for all the loci examined, and paternal isodisomy was demonstrated for multiple loci on the various parts of chromosome 14. In Patient 3, single peaks only were identified for *D14S80* and 20 loci from *D14S1069* to *D14S1007*, and paternal isodisomy was confirmed for five loci (*D14S1000*, *D14S267*, *D14S985*, *D14S1010*, and *D14S292*) on the distal part of 14q. By contrast, two peaks were found for 11 loci from *D14S1021* to *D14S981*, and biparental origin was demonstrated for seven loci (*D14S608*, five loci from *D14S75* to *D14S1026*, and *D14S1046*) on the middle to proximal part of 14q. Furthermore, the genotyping results of the remaining four loci (*D14S1021*, *D14S121*, *D14S271*, and *D14S981*) were also consistent with biparental origin, although paternal heterodisomy for *D14S1021* and *D14S981*, and maternal heterodisomy for *D14S121* and *D14S271* might theoretically be possible. Taken together, the results indicated segmental paternal isodisomy for chromosome 14 distal to *D14S981* at 14q23.3 in Patient 3.

Methylation Specific PCR Assay

Although PCR products were obtained with the methylated allele specific primers, no PCR products were yielded with the unmethylated allele specific primers in Patients 1–3 (Fig. 3). In a control subject, PCR products were obtained with both of the primers.

DISCUSSION

The results indicate that Patients 1 and 2 had full paternal isodisomy and Patient 3 had segmental paternal isodisomy for chromosome 14. The full paternal isodisomy in Patient 1 with a normal karyotype could most likely be due to duplication of